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UNIVERSITY OF ALBERTA

QUALITY ASPECTS OF CO_2 STORED BEEF STEAKS

BY

SANDRA KAYE NESOM-FLEET

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

FOOD SCIENCE

DEPARTMENT OF FOOD SCIENCE AND NUTRITION

EDMONTON, ALBERTA

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SUBMITTED BY: SANDRA KAYE NESOM-FLEET

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF:

MASTER OF SCIENCE

in

FOOD SCIENCE

Dr. F. Wolfe (Supervisor)

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March 28, 1994

DEDICATION

This thesis is dedicated to my husband Joe, for all his love and patience, and for encouraging me to be strong; and to my parents for teaching me to believe in myself and for giving me the opportunity to acheive my goals.

ABSTRACT

Controlled atmosphere (CAP) storage in 100% CO_2 was examined. Steaks from three different muscles were stored at -1.5°C for up to 24 weeks. At three week intervals, steaks were transferred to retail display for 30 hours. Colour (sensory and instrumental), discolouration, odour, pH and retail acceptance were evaluated. Sensory colour scores remained constant over the 24 week storage interval but instrumental measurements showed a significant increase in the percentage of metmyoglobin and a decrease in the percentage of oxymyoglobin as both retail display time and CO_2 storage time increased. Deoxymyoglobin was detectable only after removal from 100% CO_2. Discolouration increased, and retail acceptance and odour acceptability decreased as both retail display time and CO_2 storage time increased. The pH decreased significantly for all steaks throughout the experiment.

Microbiological changes that occured during anoxic storage and retail display were evaluated at typical (6.3°C) and ideal (2.6°C) temperatures. Steaks of one muscle type only were packaged under 100% CO_2 and stored at -1.5°C or 2.0°C for up to 24 weeks. At six week intervals, steaks were transferred to the retail environment for 10 days, and bacterial growth, colour, discolouration, retail acceptance, off-odour intensity and case-life were evaluated. Throughout the 24 week CO_2 storage intervals, the lactic acid bacteria were the only detectable microorganisms. The lactic acid bacteria also dominated each 10 day retail display interval with the pseudomonads emerging as the only other detectable group of microorganisms. Colour remained quite constant and the amount of discolouration increased with increasing time. Off-odours increased with increasing anoxic storage time and time on retail display. Case-life (days) decreased significantly with time under CO_2 storage and appearance deteriorated quicker than odour.

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Literature Review

PRESERVATIVE PACKAGING

The success of a preservative packaging system for fresh beef depends on the acceptability of the final product as well as the system's ability to protect the meat from physical and chemical damage or change, and from microbial deterioration (Muller, 1990). The meat package should hold the product, protect the product from contamination, prevent loss of moisture and allow the product to be attractive in appearance in order to entice the consumer (Goepfert, 1988). Throughout storage, microbial growth must be controlled to improve beef safety and maintain beef quality (Gill and Molin, 1991; Greer *et al.*, 1993). Once removed from the packaging system, the beef must be transferred to an aerobic atmosphere so that it can "bloom" to the bright red colour that consumer's prefer (Seideman *et al.*, 1984; Manu-Tawiah *et al.*, 1991). Also, if preservative packaging systems for fresh beef are to be effective, a desirable colour, odour, and flavour must be achieved and maintained throughout retail display (Shay and Egan, 1987).

Vacuum packaging is one form of modified atmosphere packaging (MAP) (Goepfert, 1988; Brody, 1989b). As storage progresses, meat respiration reduces the residual oxygen content in the package to much less than 1%, while carbon dioxide levels can reach 20-40% (Taylor and MacDougall, 1973; Hermansen, 1983). A good vacuum-packaging system requires the packaging film to be in close contact with all meat surfaces (Egan, 1984; Brody, 1989b). The "Cryovac" system of vacuum shrink

packaging of primal and subprimal cuts of red meat was introduced in the 1960's (Young *et al.*, 1988; Brody, 1989b).

Another form of MAP involves surrounding the meat in a preservative gaseous atmosphere (Seideman and Durland, 1984; Brody, 1989b). The meat and the gas are allowed to interact and consequently, the gaseous atmosphere composition is modified by the attainment of equilibrium with dissolved gases (Brody, 1989a). During meat storage, oxygen is consumed by respiration, as the meat mitochondria remain functional up to 144 hours post mortem, or as long as the pH remains greater than 5.5 (Cheah and Cheah, 1971). The concentration of CO_2 is decreased by dissolution in the meat liquid (Taylor and MacDougall, 1973; Gill, 1988). These changes occur because CO_2 is soluble in both water and fat (Shay and Egan, 1987; Gill, 1988). There are 2 types of MAP, high-oxygen MAP and low-oxygen MAP. High-oxygen MAP contains up to 70% oxygen and approximately 30% CO_2 (Gill, 1990). In the low-oxygen system, CO_2 levels are greater than 60% and oxygen levels are less than 40%. A combination of 80% O_2 and 20% CO_2 seems to produce the best shelf life for beef (O'Keefe *et al.*, 1975) and pork (Ordonnez and Ledward, 1977).

Controlled atmosphere packaging (CAP) is a packaging system where a specific atmosphere is applied and maintained throughout the package life (Brody, 1989b). The fresh meat is packaged with excess CO_2 in an aluminum foil laminate pouch that is impermeable to all gases (Gill, 1989). The value of CO_2 gas in food preservation systems has been known for many years (Coyne, 1932; Kraft and Ayres, 1952; Clark and Lentz, 1969; Silliker and Wolfe, 1980). As early as 1882, CO_2 was recognized as having preservative properties in fresh meats (Holland, 1980; Finne, 1982).

CO_2 absorption and formation, and ionization of carbonic acid occurs as a result of packaging in 100% CO_2 (Brody, 1989b). The success of CAP demands that the

packaging system remove all but traces of oxygen, the packaging material be completely impermeable (Seman *et al.*, 1989; Gill and Molin, 1991) and temperature control be precise. Also, the CO_2 gas must be of food grade quality and contain less than 200 ppm oxygen. For maximum storage life, sufficient CO_2 is required to maintain a CO_2 atmosphere around the meat at all times (Gill and Penney, 1988). The general rule when using a CAP system is to use 1.5 to 2.0 litres of CO_2/kg of meat (Gill and Penney, 1988). CO_2 absorption is most rapid during the first 48 hours after packaging (Hermansen, 1983). The solubility of the gas depends upon muscle pH, temperature, and fat composition and amount (Gill, 1988). An excess of CO_2 in the initial package is necessary to achieve the desired amount of gas/kg of meat after equilibration.

In the CAP system, the increased partial pressure of CO_2 causes an "antimicrobial effect" (Finne, 1982; Seideman and Durland, 1984). As the concentrations of CO_2 increase, microbial growth inhibition increases proportionately (Brody, 1989b). Anoxic conditions prevent the growth of aerobic species and the high levels of CO_2 prevent organisms tolerant to anaerobic environments from growing (Gill and Penney, 1986; Shay and Egan, 1986; Sebranek, 1986). Temperature plays a major role as lower temperatures seem to improve CO_2's inhibitory effects (Gill and Tan, 1979; Enfors and Molin, 1981; Finne, 1982). An atmosphere of 100% CO_2 can increase storage life 8-15 times that of air storage (Gill, 1990) provided that pH, temperature and initial microbial contamination are controlled (Spahl *et al.*, 1981; Gill and Penney, 1985; Gill and Penney, 1988; Gill and Harrison, 1989).

A process developed for efficient CAP of meat is called "CAPTECH", from which has evolved the "Captron" packaging machine (Warburton and Gill, 1989). The CAPTECH process can be adapted to suit retail, wholesale, domestic or international

markets; required storage lives; and the type and form of meat to be packaged (Gill, 1990). The main objective of the CAPTECH system is to produce a package containing 100% CO_2 with residual oxygen levels of less that 500 ppm. The CATPECH system is an effective controlled atmosphere packaging system for fresh, chilled and processed meats (Gill, 1989). Much research in the packaging area has shown that CAP is an excellent system for controlling micrbial growth, improving safety and dramatically increasing shelf life (Huffman *et al.*, 1975; Gill and Harrison, 1989; Greer *et al.*, 1992).

New research in the area of CAP focusses on packaging retail ready cuts of beef in 100% CO_2. The advantages of retail ready packaging of beef are: decreased labour and cost at the retail level, convenience, portion control, decreased spoilage, improved inventory control, improved quality control, and improved hygiene (Scholtz *et al.*, 1992a). Disadvantages associated with centralized packaging of retail ready cuts are increased labour at the central facility, lack of consumer acceptance to centrally pre-packaged meats and lack of retail marketing expertise (Cole, 1986).

COLOUR

a) Importance and Nature

Colour is often the consumer's basis for rejection or selection of beef (AMSA, 1991). In fact, colour is the most important factor in determining consumer acceptance (Jeremiah, 1982; Kropf *et al.*, 1985; Agullo *et al.*, 1990). Unfortunately, a bright "cherry-red" colour on the beef surface has come to represent "freshness" to the consumer (Pirko and Ayres, 1957, Adams and Huffman, 1972) and any other

colour for fresh beef is not acceptable. Therefore, it is essential that this acceptable colour be achieved and maintained throughout beef storage and retail display periods. It has been well documented that as both storage time under anoxic conditions and storage temperature increase, there is a significant decrease in retail shelf life (Gill, 1986; Shay and Egan, 1990). Shelf life is limited due to unacceptable colour changes which occur much sooner than spoilage by microorganisms (Kropf *et al.*, 1985; Moore and Gill, 1987).

Colour itself consists of the three separate dimensions; hue, value, and chroma (AMSA, 1991). Hue is the name of the colour and it is determined by the wavelength of light impacting the retina of the eye. Value describes the lightness of the colour. Finally, chroma is related to colour intensity or colour saturation. All three parameters must be evaluated together (AMSA, 1991) when evaluating beef colour. As storage under aerobic conditions increases, the beef colour changes by increasing in lightness (value), it becomes less saturated and dull (a decrease in chroma), and changes from cherry-red to greyish-brown (a change in hue) (Van Laack *et al.*, 1989). Beef colour is determined by the chemical state and concentrations of the three myoglobin forms and by the physical charcteristics of the meat itself (Kropf *et al.*, 1985; Renerre, 1990).

b) Chemistry

Meat colour chemistry is based on the two hemoproteins, hemoglobin and myoglobin (Kropf *et al.*, 1985). Hemoglobin (M.W. 67,000) is the primary pigment found in blood and it is lost during slaughter and bleeding of the animals (Seideman *et al.*, 1984). Consequently, myoglobin (M.W. 18,000) is the pigment mainly responsible for the colour of beef. For beef, the concentration of myoglobin in the muscle cells

can range from 4.0-10.0 mg/g (Bodwell and McClain, 1971; Bandman, 1987) depending on muscle type, genetic factors, exercise and diet (Kropf, 1980).

The structure of myoglobin is very complex. This pigment is a globular protein consisting of a prosthetic heme group (protoheme or proto porphyrin IX), surrounded by a single polypeptide chain of 153 amino acids (Bandman, 1987; Seideman *et al.*, 1984). The porphyrin ring encloses an iron atom situated within the plane of the four pyrrole rings (Seideman *et al.*, 1984). Four of the six coordination sites of the ferrous atom are bound by methine bridges. The fifth coordination site is bound to the imidazole nitrogen of the distal histidyl (His^{93}) residue of the polypeptide chain (Kendrew, 1961). The free (sixth) coordination site within the heme complex can be occupied by hydroxyl ions or water molecules depending on pH, oxygen, CO_2, or other electron pair donors.

The three most common chemical states of myoglobin are deoxymyoglobin, oxymyoglobin and metmyoglobin. Deoxymyoglobin is the reduced form of myoglobin, with the heme iron in the ferrous (Fe^{2+}) state (Kropf *et al.*, 1985). It has a dark red to purplish-red colour, can bind to water and occurs when oxygen partial pressures are very low (less than 1.4 mm) (Kropf *et al.*, 1985; Bandman, 1987).

When oxygen partial pressures reach levels of 25 mm or higher, oxygen and the ferrous heme iron will rapidly combine to form oxymyoglobin (Kropf *et al.*, 1985). It has a bright red colour. Oxymyoglobin has one mole of O_2 bound to every mole of ferrous heme iron and this reaction is completely reversible. Oxygenation of myoglobin will only proceed when iron is in the ferrous state (Livingston and Brown, 1981). If conditions arise that produce low oxygen tensions (6 +/- 3 mm Hg at 0°C) (Ledward, 1970) the oxidized form of myoglobin, metmyoglobin, will form.

Metmyoglobin has iron in the ferric (Fe^{3+}) state and is an undesirable brown colour. Oxidation is the term used to describe the change in iron from the Fe^{2+} state to the Fe^{3+} state. Molecular oxygen dissociates from the heme, removing one electron from the ferrous iron (Giddings and Markakis, 1973). An individual muscle can have a wide range of oxygen partial pressures allowing all three pigment forms to be present at any one time. Deoxymyoglobin, oxymyoglobin and metmyoglobin are continuously interconverting and their reactions are completely reversible (Clydesdale and Francis, 1971; Kropf *et al.*, 1985).

When a cut of fresh beef is sliced perpendicular to the surface, three separate and distinct colours can be seen (Taylor, 1972). The outer surface of the beef shows the bright cherry-red colour of oxymyoglobin due to the exposure of oxygen. The inner-most layer will be the purplish-red colour of deoxymyoglobin due to the absence of oxygen. Between these two layers, a thin layer of brown metymoglobin often forms because of the low oxygen tensions (Taylor, 1972). When beef is freshly cut, oxygen will penetrate the tissue producing a cherry red colour for several mm, because myoglobin has a very high affinity for oxygen (Pirko and Ayres, 1957). This is commonly referred to as "blooming" and takes about one-half hour (Johnson, 1974) after oxygen exposure.

Oxygen will continue to penetrate into the beef tissue for several days, depending on the storage conditions. For example, colder temperatures (0°C) produce greater oxygen penetration than higher temperatures. Increased temperatures favour increased respiratory enzyme activity, and this consumes oxygen (Kropf *et al.*, 1985). Muscle tissue will acccumulate oxymyoglobin quickly and at a greater depth if the atmosphere surrounding the muscle has a high oxygen partial pressure (Taylor, 1982). Eventually oxymyoglobin will start to move back towards the surface, allowing the

thin metmyoglobin layer to also rise to the surface and become thicker (Kropf *et al.*, 1985). The extent of metmyoglobin accumulation on the surface of fresh beef depends on a number of interdependent factors. Regardless of the causes, metmyoglobin formation is not desirable. The consumer will reject beef if it shows as low as 20% metmyoglobin on the surface (MacDougall, 1982; Renerre and Mazuel, 1985).

Oxidation of oxymyoglobin to metmyoglobin is caused by any condition that reduces the oxygen tension at the beef surface, which results in oxygen disassociating from the heme complex of oxymyoglobin. Some factors influencing metymoglobin formation are pH (Brooks, 1937; Walters, 1975; MacDougall, 1982; Lawrie, 1985; Renerre, 1990), high temperatures (Lawrie, 1974; Walters, 1975), light (Lawrie, 1974), low oxygen atmospheres (Ledward, 1970), aerobic bacteria (Butler *et al.*, 1953; Robach and Costilow, 1962), muscle type (Ledward, 1971; MacDougall and Taylor, 1975; O'Keefe and Hood, 1980-81b; Renerre and Labas, 1987), lipid oxidation (Watts, 1962; Ledward, 1983), oxygen consumption rate (Bendall and Taylor, 1972; O'Keefe and Hood, 1982; Ledward, 1985) and metmyoglobin reducing activity (MRA) (Giddings, 1974; Seideman *et al.*, 1984; Kropf *et al.*, 1985; Ledward, 1985).

c) Effect of pH

The pH of beef after rigor resolution (usually 2-3 days post slaughter) is close to the isoelectric point of beef's structural proteins (5.5) (Dainty *et al.*, 1983), between 5.6 and 5.8 (Cole, 1986). Stress immediately before slaughter can result in an accumulation of lactic acid in the muscle tissue causing pH to decline. Decreased pH opens the muscle fibril structure, increasing light scattering and causing an overall paler colour (Walters, 1975; Kropf *et al.*, 1985). This open structure condition is

referred to as PSE (pale, soft, exudative) meat and can occur in pork (Renerre, 1990) but seldom in beef (Hunt and Hedrick, 1977). Also, it has been shown that at low pH (below pH 5.4) (Cole, 1986), oxidation is accelerated (Livingston and Brown, 1981) by the denaturation of oxymyoglobin's globin moiety. This facilitates the conversion of oxymyoglobin to deoxymyoglobin (Walters, 1975), which then readily oxidizes to metmyoglobin (Pirko and Ayres, 1957).

In beef, pre-slaughter stress often results in high pH meat (greater than 5.8) that is dark-purple in colour and will not bloom when exposed to air (Egbert and Cornforth, 1986). High pH beef is more susceptible to putrefactive microbial spoilage than normal pH beef (Gill and Newton, 1980; MacDougall, 1982). At increased pH, muscle mitochondrial respiratory activity increases (Egbert and Cornforth, 1986), and the surface layer of oxymyoglobin is very thin. Therefore, the purplish-red colour of deoxymyoglobin is more apparent (Renerre, 1990) making the meat seem darker in colour.

d) Effect of Temperature

High temperatures cause the globin of oxymyoglobin to lose it's role of protecting the heme against deoxygenation (Seideman *et al.*, 1984) and will produce deoxymyoglobin which is quite unstable (Walters, 1975). When the heme is unprotected the oxidation of deoxymyoglobin occurs spontaneously. As stated previously, high temperatures increase the activity of oxygen-utilizing enzymes within the muscle tissue (Lawrie, 1974; Kropf *et al.*, 1985), as well as other oxygen-using processes such as fat oxidation (Renerre, 1990). Elevated storage and display temperatures (above 1.67°C) tend to decrease the depth that oxymyoglobin penetrates

the beef tissues (Brooks, 1937), allowing the metmyoglobin layer to move closer to the meat surface. Therefore, as display time increases, the amount of visible metmyoglobin increases. The muscle's reducing activity as well as the gaseous environment are also influenced by temperature (Renerre, 1990), as high temperature has been shown to increase reducing activity and may decrease metmyoglobin formation in anaerobic systems (Renerre and Labas, 1987).

e) Effect of Light

It has been well documented that light has a profound effect on the rate of metmyoglobin formation (Kropf, 1980; Klettner, 1984; AMSA, 1991) but there are conflicting reports which conclude that light has no effect on muscle discolouration (Kropf, 1980). Light reaching the beef surface is either absorbed, reflected (Hunt, 1980) or scattered but rarely transmitted (AMSA, 1991). Therefore, the type of light and intensity will effect the amount of autoxidation. Kraft and Ayres (1954), Watts (1954), and Clauss *et al.*, (1957) found that no differences could be found in the colour of fresh meat stored in the light or in the dark. Jeremiah *et al.*, (1972), found that lamb roasts had a darker colour when stored in the light, but vacuum-packaged rib chops had colour improvements when subjected to light storage. Studies by Marriott *et al.*, (1967) and Bala and Naumann (1977) conclude that light causes a significant colour change characterized as unacceptable discolouration. A review (Kropf, 1980), of the effects of retail case lights on beef colour concluded that light does have an effect on pigment state as well as overall colour but the human eye may not be sensitive enough to detect any such differences. A more recent review (Renerre, 1990) suggests that light does play a significant role in pigment oxidation and

metmyoglobin formation in fresh, cured, and frozen meats . Obviously, light has an effect on different meat types.

The light source is important when evaluating beef discolouration, as different lights can cause differences in the amount of meat surface heating, colour rendition (caused by differences in spectral energy patterns) and colour stability (Kropf, 1980). In the visible spectrum, oxymyoglobin has absorption peaks at 545 and 582 nm, deoxymyoglobin shows a broad absorbance spectrum with a maximum at 555 nm, while metmyoglobin has absorption bands at 505 and 630 nm (Francis and Clydesdale, 1975). Incandescent lighting can produce a radiating effect by raising the surface temperature of the beef. Light from display systems can increase the surface temperature of the beef by 2 to 3°C (Powell and Cain, 1987) because heat can get trapped between the packaging film and the beef surface. Warmer temperatures at the surface promote metmyoglobin formation (Kropf, 1980; Renerre, 1990).

White flourescent lights do not seem to cause any significant colour changes (Renerre, 1990), and the use of "natural" rather than "cool white" fluorescent lights is preferred. "Natural" lights bring out the red colour of beef and suggest that the product is fresh (Klettner, 1984). The closer the light source is to the typical reflectance pattern of the muscle, the more appetizing the muscle will appear (Clark, 1956). Flourescent lighting is also beneficial for producing the desired light intensity, as too high an intensity can decrease the display life of gas-packaged and frozen meats (Kropf, .1980). UV light is undesirable because myoglobin oxidizes quickly to metmyoglobin with only a short exposure, possibly due to globin denaturation (Lawrie, 1985).

f) Effect of Packaging System

Packaging systems and packaging films play a significant role on pigment concentration and beef colour (Kropf, 1980; Seideman *et al.*, 1984). All packaging types must prevent the beef from contamination, act as a moisture barrier and prevent evaporation (Brody, 1989a; Renerre, 1990).

Vacuum-packaged beef can discolour within the pack itself if the beef is not in close contact with the film (Rizvi, 1981; Egan, 1984; Egan and Roberts, 1987). Any areas having vacuities will accumulate oxygen as storage intervals increase (Gill, 1990). Packages having an oxygen partial pressure between 6-7.5 mm Hg (George and Stratmann, 1952; Ledward, 1970) will promote formation of the brown pigment metmyoglobin (O'Keefe and Hood, 1982). Another colour problem with vacuum-packaged beef is that the anoxic conditions favour the development of purplish-red deoxymyoglobin (Shay and Egan, 1987). Within 48 hours of storage time any residual oxygen is consumed and CO_2 accumulates. Oxymyoglobin diminishes and metmyoglobin gradually moves towards the surface (Kropf *et al.*, 1985). Deoxymyoglobin will ultimately form if the muscle has sufficient reducing capacity (Kropf *et al.*, 1985). Unfortunately, consumers feel that any deviation from the bright red of oxymyoglobin is an indication of product deterioration or poor quality(Kropf *et al.*, 1985; Manu-Tawiah *et al.*, 1991). Due to these colour problems, vacuum-packaging is not recommended for small cuts and display packs of red meat (Young *et al.*, 1988; Allen, 1989). However, vacuum-packaging is successful for storing primal cuts of normal pH beef at temperatures below +1.0°C for up to 10 weeks (Seman *et al.*, 1988; Gill and Penney, 1988).

The main advantage of packaging fresh beef in MAP containing oxygen is that a thick layer of oxymyoglobin is produced (Renerre, 1990). High-oxygen MAP can double colour stability and increase the time to reach spoilage (Gill, 1990) as there is both a colour-enhancing effect as well as a bacteriostatic effect (Clark and Lentz, 1972). This can only be achieved if the pack volume is three times that of the meat volume. Unfortunately, the increased space requirement is a disadvantage to the beef industry (Taylor, 1985).

MAP oxygen levels greater than 50% produce beef with less metmyoglobin formation (Ordonez and Ledward, 1977; Silliker *et al.*, 1977; Renerre, 1990) and the best colours are produced in MAP containing oxygen concentrations greater than 60% (Taylor, 1985). High-oxygen MAP is not recommended for prolonged storage of beef as the accompanying moderate CO_2 levels decrease the growth of aerobic spoilage organisms but do not completely inhibit their growth (Gill, 1990). Also, increased oxygen levels in the atmosphere tend to cause increased off-odours and rancidity in the beef, probably due to lipid oxidation (Taylor, 1985; Renerre, 1990). In low-oxygen MAP systems, deoxymyoglobin is readily oxidized to metmyoglobin (Ledward, 1985) and red meats tend to discolour very quickly due to the low oxygen levels (Gill, 1990). Therefore, red meats cannot be successfully packaged in low-oxygen MAP (Hotchkiss *et al.*, 1985; Renerre, 1990).

In the CAPTECH system there is no oxygen, therefore no oxidation reactions occur. Beef packaged in 100% CO_2 is essentially stable and the pigment is predominantly in the deoxymyoglobin form (Gill, 1990). A major problem with a system using 100% CO_2 is that the colour of beef is very unstable once transferred to an aerobic environment (Finne, 1982; Taylor *et al.*, 1990). Extended anoxic storage of chilled beef causes retail acceptability to decrease under aerobic conditions (Moore

and Gill, 1987; Shay and Egan, 1990). However, as long as there is careful control of the transfer to air, and temperature and lighting are controlled, this reduction in shelf life should still permit the beef to be displayed for up to 2-3 days, all that is required in normal retail markets.

The need to exercise the above mentioned controls may cause serious problems for retailers, but can be overcome by centralized preparation of the beef in retail ready consumer packs (Cole 1986; Gill, 1990). Individual steaks can be placed on polystyrene trays, flushed with the desired gas and sealed with a film of high oxygen-permeability. These individual trays can be placed inside a larger master pack. The master package is evacuated and is then filled with the desired gas and heat sealed (Sheridan, 1988; Scholtz *et al.*, 1992a). During storage the gas in the master pack diffuses into the retail ready packs (Cole, 1986). The CAP system keeps functioning until the master pack is opened just prior to the steaks being placed onto retail display (Gill, 1990).

As previously mentioned, CO_2's beneficial effects are temperature dependent, and storage conditions must be below 5°C to produce maximum gas solubility within the meat (Renerre, 1990). CO_2 can have a residual effect on the beef after the CAP system is opened (Renerre, 1990), as CO_2 inhibition continues to be expressed as demonstrated in studies where pork chops are transferred to the aerobic conditions of retail display (Spahl *et al.*, 1981; Scholtz *et al.*, 1992a; Scholtz *et al.*, 1992b).

For retail display and consumer selection, beef is often overwrapped in polyvinyl chloride films that have a high degree of oxygen permeability (Seideman *et al.*, 1984, Cole, 1986). This type of film allows the beef to "bloom" to the desired cherry-red colour of oxymyoglobin (Penney and Bell, 1993). Landrock and Wallace (1955) recommend that the film have an oxygen permeability rate of at least 5 cc/m^2/24 hours

to prevent conversion of oxymyoglobin to metmyoglobin. As storage time on retail display increases the demand for oxygen by oxygen-utilizing enzymes increases (Powell and Cain, 1987). This causes low oxygen tensions and results in metmyoglobin formation (Clydesdale and Francis, 1971; Shay and Egan, 1987). The main factor affecting colour and shelf life of beef is temperature (Greer and Jeremiah, 1980; Powell and Cain, 1987). Low temperatures slow the conversion of oxymyoglobin to deoxymyoglobin and inhibit bacterial growth which may also lead to discolouration (Powell and Cain, 1987). The recommended display temperature range is 0 to 3°C (Powell and Cain, 1987), and a good rule of thumb is: for each 2°C of storage temperature increase there is a 1 day loss in case life.

g) Effect of Microorganisms

The growth of some microorganisms causes beef to be unacceptable due to changes in appearance, odour and flavour (Dainty *et al.*, 1983; Gill, 1986) but colour itself is not an indicator of bacteriological or overall eating quality (Egan and Roberts, 1987). The high amount of moisture inside meat packages provides ideal conditions for microbial growth (Renerre, 1990). Aerobic bacteria have been related to metmyoglobin formation (O'Keefe and Hood, 1982; Seideman *et al.*, 1984), which seems to occur when the bacteria are in the logarithmic phase of growth (Butler *et al.*, 1953). Psychrotrophic, aerobic organisms such as *Achromobacter*, *Pseudomonas*, and *Flavobacterium* may contribute to undesirable browning by decreasing the oxygen available to the beef surface (Jay, 1972; Dainty *et al.*, 1983; Renerre, 1990).

The lactic acid bacteria, common flora of vacuum-packaged and CAP meats, do not contribute to any significant colour changes (Robach and Costilow, 1962; Hanna

et al., 1983; Renerre, 1990). However, certain other bacteria, in combination with high pH, can generate metabolic by-products, for example hydrogen peroxide and hydrogen sulfide, that act as oxidizing agents (Kropf *et al.*, 1985; Renerre, 1990). Hydrogen peroxide oxidizes the heme moiety of unstable deoxymyoglobin producing the green pigment choleglobin (Walters, 1975; Seideman and Durland, 1984). Hydrogen sulfide can oxidize deoxymyoglobin and produce the green pigment sulphmyoglobin (Walters, 1975; Seideman and Durland, 1984). Sulphmyoglobin seems to be a major problem in vacuum-packaged, high pH meat (Breidenstein, 1982; Renerre, 1990). The lactic acid bacteria may actually be important in improving the colour stability of certain meats packaged in high CO_2 by controlling the growth of potent spoilage organisms (Shay and Egan, 1986; Gill and Harrison, 1989) and improving beef colour characteristics (Renerre and Montel, 1986).

h) Effect of Muscle Type

Individual muscles can differ in their colour stability (O'Keefe and Hood, 1982; Renerre, 1990) and there are many reasons why this occurs (Faustman and Cassens, 1990). The function of the muscle and the fiber types it contains determines its myoglobin content (Seideman *et al.*, 1984; Renerre and Labas, 1987). The three major fiber types are slow-twitch oxidative (red), fast-twitch glycolytic (white) and fast-twitch oxidative-glycolytic (intermediate) (Renerre, 1990). Slow-twitch oxidative fibers are used for slow, long-lasting contractions that require aerobic metabolism. These fibers have high enzymic activity, a large number of mitochondria, are present in high concentrations in "red" muscle (Renerre, 1990) and require oxygen which is carried by myoglobin (Seideman *et al.*, 1984).

This "red" muscle (beef) is more susceptible to oxidative rancidity as it has a higher percentage of lipid as compared to "white" muscle (poultry) (Renerre, 1990). Oxidative rancidity will effect the amount of discolouration during storage (Renerre, 1990). Fast-twitch glycolytic fibers contract readily but tire quickly (Seideman *et al.*, 1984). The white fibers metabolize anaerobically and therefore do not require oxygen (Seideman *et al.*, 1984). Fast-twitch glycolytic fibers are low in mitochondria but high in glycogen and phosphates (Renerre, 1990).

Unfortunately, differences in colour stability between different beef muscles can not be entirely explained by evaluating fiber type alone (Renerre, 1990). Fiber types can vary among and within muscles (Hunt and Hedrick, 1977). Also, muscles have different pigment concentrations and this is difficult to control (Kropf, 1980). The physical characteristics of the animals such as age, sex, nutritional regime, and antemortem and postmortem treatment all can effect the colour pigments (Kropf, 1980).

The concentration of NADH within the muscle appears to play a major role in colour stability (Echevarne *et al.*, 1990). NADH catabolism is slower in stable muscles (*Longissimus dorsi*) and quicker in less stable muscles (*Diaphragma medialis*) (Echevarne *et al.*, 1990). Storage conditions can effect the colour stability once the meat is transferred to retail display. After storage in oxygen-free gas atmospheres, the colour shelf life is related to the differences in intrinsic properties of different muscles (O'Keefe and Hood, 1980-81a) and decreases as storage time increases.

i) Lipid Oxidation

Lipid oxidation is one of the major causes of beef quality deterioration during refrigerated storage (Alford *et al.*, 1971; Raharjo and Sofos, 1993) and myoglobin may be one of the lipid oxidation catalysts (Ledward, 1983). The process of lipid oxidation is a chain reaction consisting of several initiation, propagation, and termination reactions (Renerre, 1990). Polyunsaturated fatty acid hydroperoxides degrade and can form a number of secondary products including malonaldehyde (Raharjo and Sofos, 1993). The TBA test is the most common test to measure malonaldehyde concentration (Raharjo and Sofos, 1993).

The rate of lipid oxidation depends on the oxygen tension, temperature, storage conditions, and fat composition (Gill, 1982). For example, some microorganisms produce lipases, which cause lipolytic changes that increase myoglobin oxidation to metmyoglobin (Govindarajan *et al.*, 1977; Bala *et al.*, 1979). Vacuum packaging seems to slow lipid oxidation in fresh beef (Genigeorgis, 1985; Fu *et al.*, 1992). Certain reductants have been shown to delay lipid oxidation (Manu-Tawiah *et al.*, 1991). A combination of tetrasodium pyrophosphate, sodium erythorbate and citric acid added to ground beef lowered TBA values and increased colour shelf life (Manu-Tawiah *et al.*, 1991). Unfortunately, there is no real evidence to prove that heme pigment oxidation initiates lipid oxidation (Renerre, 1990) as there is disagreement on what form of iron causes the catalytic effect (Ledward, 1983; Renerre, 1990). Also, because the heme is sterically hindered within the myoglobin molecule it would be difficult for other ligands to reach it (Giddings, 1977).

j) Effect of Muscle Respiration

The oxygen consumption rate of mitochondria plays an important role in metmyoglobin formation and colour stability by promoting a narrow oxymyoglobin layer on the beef surface and allowing metmyoglobin and dexoxymyoglobin to be more visible (Renerre and Labas, 1987). Oxygen consumption rate is one of many factors involved in beef colour changes (Lanari and Cassens, 1991) early post mortem (Ledward, 1985), because the brightness and saturation of the red colour is dependent upon the depth to which oxygen can penetrate the muscle tissue and oxygenate deoxymyoglobin (Atkinson *et al.*, 1969). The depth of oxygen penetration increases as oxygen consumption rate decreases (O'Keefe and Hood, 1982). Muscles that have a high rate of oxygen consumption also have the poorest colour stability and fastest rates of metmyoglobin formation (O'Keefe and Hood, 1982; Renerre and Labas, 1987).

Beef that is in the oxymyoglobin form will continue to consume oxygen and remain bright red in colour as long as aerobic conditions persist (Hermansen, 1983). Muscle mitochondria consume 1-3 μl of oxygen/cm^2 every 24 hours (Cheah and Cheah, 1971). Mitochondrial ability to consume oxygen is most pronounced during the first 48 hours post slaughter, and then declines as mitochondrial enzymes are deactivated (Hermansen, 1983). Oxygen consumption rate decreases with the age of the beef post mortem due to the degredation of mitochondrial enzymes, substrates and coenzymes (Atkinson *et al.*, 1969; De Vore and Solberg, 1974), high temperatures and low pH (Ledward, 1985). Oxymyoglobin is gradually transformed into metmyoglobin as storage time increases. As mentioned previously, low storage temperatures decrease the activity of oxygen-utilizing enzymes helping to keep the oxymyoglobin form

(Lawrie, 1974; Seideman *et al.*, 1984). High ultimate pH favours increased oxygen consumption rate and produces purplish-red, dark coloured meat (Renerre, 1990).

h) Effect of Reducing Systems

Some researchers have found that the activity of the enzymic reducing system is an important factor in metmyoglobin formation in beef muscle (Ledward, 1985), while others conclude that the activity of the reducing system does not explain differences in colour stability and metmyoglobin formation (O'Keefe and Hood, 1982; Renerre and Labas, 1987; Faustman and Cassens, 1990). However, the exact nature of the system or the reactant(s) involved is not clear (Ledward, 1985), and the presence of a specific reductase in muscle has not been proven convincingly (Echevarne *et al.*, 1990). Natural metmyoglobin reductase systems were first discovered in 1960 by Dean and Ball and these reductases have been isolated from beef heart (Hagler *et al.*, 1979).

Meat is a biological material that contains enzyme systems that consume oxygen and produce carbon dioxide (Powell and Cain, 1987). These enzyme systems are able to convert metmyoglobin back to deoxymyoglobin which immediately gets oxygenated back to oxymyoglobin (Kropf *et al.*, 1985). This process is referred to as "metmyoglobin reducing activity" or "MRA" (Seideman *et al.*, 1984). The reducing enzyme system is able to donate an electron to the iron molecule changing iron from the ferric to the ferrous state (Seideman *et al.*, 1984). Unfortunately, beef has only a limited capacity to convert metmyoglobin back to deoxymyoglobin and when metmyoglobin production exceeds the enzymic reducing system of the beef (which takes approximately 168 hours depending on storage conditions) the meat surface will become permanently brown (Penney and Bell, 1993).

Some muscles remain a bright red colour longer than others due to their higher MRA (Seideman *et al.*, 1984). Darker muscles (*psoas major*) that have poor colour stability tend to use up their MRA quickly and will discolour to brown metmyoglobin (O'Keefe and Hood, 1982). *Longissimus dorsi* and *semimembranosus* muscles have a less active reducing system and therefore are more colour stable (Echevarne *et al.*, 1990). MRA can be affected by time, temperature, and pH (Stewart *et al.*, 1965; Ledward, 1985). The ability for metmyoglobin to be converted back to deoxymyoglobin by this enzyme system decreases with time postmortem (Atkinson and Follett, 1973), decreases as temperature increases (Stewart *et al.*, 1965) and increases with higher pH (maximum activity being above pH 5.8) (Stewart *et al.*, 1965).

The loss of MRA is also due to the decrease in function and structural integrity of the muscle mitochondria (Giddings, 1974). The exact location of these reducing enzymes is still under question (Echevarne *et al.*, 1990), but the highest activity appears to be localized in the microsomes and mitochondria (Echevarne *et al.*, 1990). Allowing beef muscle to bloom to form oxymyoglobin results in a gradual reduction in MRA. Therefore, if beef is to be stored under anoxic conditions, exposure time to oxygen should be minimized before packaging and retail display (Kropf *et al.*, 1985).

The MRA is used as an indicator of pigment reducing capacity and it's measurement involves pigment oxidation by potassium ferricyanide (Faustman and Cassens, 1990). There are no differences in metmyoglobin reduction measured aerobically or anaerobically (Ledward, 1985; Echevarne *et al.*, 1990) so oxygen itself does not seem to be involved. Numerous studies have concluded that NAD or NADH is a very important coenzyme involved in MRA (Giddings, 1977; Renerre and Labas, 1987; Renerre, 1990) but the NADH source is still not known (Renerre, 1990). At 6

hours post mortem NAD concentration is lower in slow-twitch red muscle fibers than in fast-twitch muscle fibers but NAD appears to equilibrate after 168 hours post mortem (Renerre, 1990). Measuring NADH concentration seems to be a more accurate indicator of muscle stability (Renerre, 1990) than pigment reduction, as pigment reduction in the presence of the artificial electron carrier methylene blue is more successful with NADH than NAD (Renerre, 1990). NADH is a limiting factor in the reduction reaction as NADH catabolism is more rapid in unstable muscles compared to more stable muscles such as *Longissimus dorsi* (Echevarne *et al.*, 1990).

Recent research has shown that MRA is not a good indicator of muscle colour stability and beef shelf life (Faustman and Cassens, 1990; Lanari and Cassens, 1991). A reducing system is present in beef but the effectiveness of MRA in colour stability and aerobic storage needs to be proven (Renerre, 1990). All of the factors contributing to metmyoglobin formation and colour stability need to be evaluated, carefully understood and carefully controlled in order to provide the meat industry with beef possessing a desirable colour and useful shelf life (Renerre, 1990).

SENSORY ANALYSIS

Product appearance has a direct influence on the consumers's decision to purchase (Stone *et al.*, 1991). Clearly, it would not be practical to package retail ready steaks under CAP for extended periods of time only to have them deteriorate very rapidly once transferred to the retail environment. Often the beef consumer's ideas on quality are quite different than the beef producer's (Stone *et al.*, 1991). Consumers often relate product quality to the visual attributes of colour and appearance (Stone *et al.*,

1991) and if the beef discolours rapidly, it will not be purchased. Odour also seems to affect consumer acceptance of fresh beef as odour can often become objectionable before the sample is rejected based on appearance (Spahl *et al.*, 1981).

The determining factor of a preservative packaging system's success is consumer acceptance and this acceptance can be determined by evaluating eating quality (Jeremiah, 1982). Advances in meat processing and preservation techniques have allowed consumers to have an increased selection of products that have very long shelf lives (Lambert *et al.*, 1991). Consequently, the average consumer has an increased awareness of and sensitivity to discolouration, off-odours and off-flavours (Lambert *et al.*, 1991).

With CAP beef, the development of acidic fermentation products from the lactic acid bacteria can be a major concern in terms of meat flavour and flavour acceptability (Egan and Shay, 1982; Dainty and Mackey, 1992). The products of the hexose fermentation pathway of the lactic acid bacteria seem to contribute to flavour development in the end product. Homofermentative lactics seem to produce a more sour taste due to the production of lactic acid only, compared to the heterofermentative types that can produce a variety of end products (Zuniga *et al.*, 1993).

The addition of lactic cultures to ground beef produced final cooked products that were preferred over control samples (no lactic cultue added) (Hanna *et al.*, 1980). Flavour scores declined for the seven day storage period for all samples but the control ground beef showed the greatest decline (Reddy *et al.*, 1970). This decline was less significant in the lactic culture-treated samples (Reddy and Chen, 1975). On the other hand, a study by Smith *et al.*, 1980, found that when steaks were inoculated with *Lactobacillus* species before vacuum storage, they had the highest incidence of off-

odour, the most surface discolouration, least desirable overall acceptance and the lowest palatability ratings.

Extended storage in 100% CO_2 should eliminate any oxidation reactions (Gill, 1990). Increasing the amounts of CO_2 progressively diminishes the development of putrid spoilage bacteria and can delay spoilage for up to 21 weeks (Gill and Penney, 1988). At this time off-flavours were described as sour-acid rather than putrid (Gill and Penney, 1988). If small amounts of oxygen (0.1, 0.2 or 1.0%) do accumulate in the controlled-atmosphere package, the odour, flavour and colour of meat (beef, pork and lamb) do not seem to be negatively affected (Penney and Bell, 1993). The CAP system seems to reduce volatile odour and flavour components and this can be an advantage with fresh meats that may have offensive odours to some consumers (Gill and Penney, 1988). A disadvantage with the CAP system is that the beef texture may be altered due to CO_2 dissolution and increased exudate (O'Keefe and Hood, 1980-81a; Gill and Penney, 1986).

There is limited information regarding the sensory properties of retail ready beef steaks after extended storage in controlled atmospheres. Vacuum packaging of retail ready pork for extended periods of time produced chops that were unacceptable in odour and flavour (Vrana *et al.*, 1985). The authors recommended that pork chops be fabricated from fresh loins and not from loins that have been vacuum packaged (Vrana *et al.*, 1985). Pork cuts stored under CO_2 were quite acceptable and during prolonged storage there was no significant decreases in texture, flavour (Gill and Harrison, 1989) and overall acceptability (Scholtz *et al.*, 1992a).

BACTERIOLOGY

a) Meat Substrates and Bacterial Growth

Meat is defined as "the edible part of the skeletal muscle of an animal that was healthy at the time of slaughter" (Canada Food and Drug Act, 1990). Fresh beef can be spoiled by microbial growth and the nature and amount of microbial contamination play an important role in the type of spoilage and storage life of the beef (Nottingham, 1982; Gill, 1982). Red meat carcasses usually carry a very diverse initial flora at a level between 10^2 to 10^6 bacteria/cm^2 (Dainty and Mackey, 1992). Beef develops a characteristic flora dependent on temperature, pH and storage atmosphere (Dainty and Mackey, 1992). Spoilage describes a wide range of conditions and has been defined as any microbial activity that changes meat flavour, appearance or odour (Gill, 1986). The concept of spoilage is important as it acts as a marker which can prevent the consumption of foods that may be dangerous to eat (Dainty and Mackey, 1992).

The most important factor in the keeping quality of fresh beef is microbial growth (Lambert *et al.*, 1991). Beef is susceptible to microbial spoilage as its chemical composition presents a rich medium for microbial growth (Gill, 1986). The composition of muscle is water (75%), protein (18-21%), lipid (3-6%), carbohydrate, and minor components such as vitamins, pigments, flavour compounds and enzymes (Gill, 1982; Lambert *et al.*, 1991). After death the muscle undergoes dramatic physical changes with the development of rigor. During rigor development there are large decreases in high energy phosphate compounds such as adenosine triphosphate (ATP) and creatine phosphate (CP), and glycogen is degraded to lactic acid causing the pH to decrease (Gill, 1982). Without ATP the muscle becomes inextensible and

rigid due to the formation of irreversible cross-linkages between actin and myosin (Huxley, 1972). The concentrations of protein or lipid do not change during the development of rigor nor are they used for growth substrates by microorganisms prior to spoilage (Dainty *et al.*, 1975; Gill, 1986).

The preferred substrates of spoilage flora are glucose, glucose-6-phosphate, lactate and amino acids (Greer, 1988). Glucose is usually the first substrate utilized by spoilage bacteria during aerobic storage (Gill and Newton, 1978; Egan and Roberts, 1987; Greer, 1988). For example, strains of *Pseudomonas* have an initial glucolytic growth phase (Egan and Roberts, 1987) and when they reach high numbers (10^8 cm^2) glucose becomes depleted and the bacteria start to use amino acids, producing malodourous sulfides, acids and esters (McMeekin, 1982). When the preferred substrates are depleted, enterics and pseudomonads can also utilize lactate for growth (Egan and Robert, 1987; Greer, 1988).

Temperature is the single most important environmental factor influencing bacterial growth on beef (Ayres, 1960; Lambert *et al.*, 1991) as growth rate declines as temperature decreases until growth finally stops (Kraft, 1986). A reduction in temperature causes an increase in the lag period and an increase in the generation time (Ingram and Mackey, 1976). Meat spoilage or food-borne disease can be caused by organisms that are capable of growing at refrigeration temperatures, <5°C. The main organisms of concern to beef spoilage are the psychrotrophs.

Psychrotrophic organisms can grow at temperatures close to 0°C, but have optimum growth temeperatures that can be 10-30°C higher (Eddy, 1960). The minimum temperature for growth of psychrotrophic meat spoilage bacteria is -3°C (Gill and Molin, 1991). The optimum storage temperature for packaged fresh beef is -1.5°C. This temperature provides the maximum microbial shelf life (Gill and Molin,

1991). Psychrotrophs can be Gram-positive or Gram-negative, aerobes or anaerobes, motile or nonmotile, and spore formers or non-spore formers (Olson and Nottingham, 1980). The composition of the microbial flora also depends on temperature as the fastest growing species become the dominant spoilage microorganisms (Lambert *et al.*, 1991).

b) Aerobic Spoilage

The most important aerobic spoilage psychrotrophs of beef are the pseudomonads (Gill, 1986; Kraft, 1986; Greer, 1988) which can account for 50-90% of the flora (Dainty and Mackey, 1992). In fact, spoilage itself is related to the growth of the pseudomonads and begins when they reach $10^6/cm^2$ (Nottingham, 1982). The pseudomonads are strictly aerobic, Gram-negative, motile rods. This genus is catalase-positive, arginine-positive and oxidase-positive, utilize sugars by oxidation and do not produce gas (Gill and Greer, 1992). Pseudomonads have a growth rate advantage and can successfully compete with other organisms at low storage temperatures (Greer, 1988; Lambert *et al.*, 1991). These organisms can cause surface discolouration by enhancing metmyoglobin formation when they reach 10^6 bacteria/cm^2 and offensive off-odours when bacterial numbers reach 10^7 to 10^8 bacteria/cm^2 (Greer, 1988).

Off-odour formation seems to be the most important indicator of the growth and metabolic activities of spoilage organisms (Greer, 1988). The putrid smell of the malodourous compounds produced from amino acid metabolism is a good indicator of aerobic spoilage (Gill, 1986). The pseudomonads can produce "fruity" odours in the initial stages of growth by producing methyl and ethyl esters of acetic, butyric and

hexanoic acids (Egan and Roberts, 1987). When high numbers of bacteria are reached, some strains of pseudomonads can produce dimethyl sulphide and putrescine (from ornithine), sulfides (from cysteine) and methanethiol (from methionine) (Egan and Roberts, 1987; Greer, 1988). The pseudomonads are capable of slime formation at maximum cell density (10^9 bacteria/cm^2). Aerobic growth stops at this point as oxygen can no longer diffuse through the slime to support bacterial growth (Kropf *et al.*, 1985; Gill and Greer, 1992).

The *Acinetobacter/Moraxella* group commonly form a large part of the aerobic spoilage flora of red meats and meats packaged in oxygen-permeable films (Gardner, 1981; Gill, 1986). The *Acinetobacter* species are strictly aerobic Gram-negative cocci or coccobacilli which are non-motile. This group of bacteria is catalase-positive, oxidase-negative and arginine-negative (Gill and Greer, 1992). The *Moraxella* genus are also strictly aerobic Gram-negative cocci or coccobacilli (sometimes occuring in pairs) which are non-motile. These bacteria are catalase-positive, oxidase-positive and arginine-negative. The *Moraxella* does not metabolize sugars and the *Acinetobacter* may utilize sugars by oxidation or not at all (Gill and Greer, 1992).

The importance of the *Acinetobacter/Moraxella* group is that they use amino acids for growth without producing malodourous by-products from amino acid degradation and therefore have low spoilage potential compared to the pseudomonads (Lerke *et al.*, 1965; Lambert *et al.*, 1991 and Gill and Greer, 1992). This group may actually enhance the spoilage potential of the pseudomonads and *Alteromonas putrefaciens* by restricting the availability of oxygen to these strictly aerobic organisms (Gill, 1986; Lambert *et al.*, 1991).

Vacuum-packaging is used extensively in the beef industry to extend the shelf life and keeping quality of fresh meat (Lambert *et al.*, 1991). Changes occur in the gas

phase of the package due to gas tranfer through the film, as well as the rate of oxygen-consumption by the beef or by facultative and/or aerobic bacteria (Nottingham, 1982). After gas equilibration, the package interior will contain <1% O_2 (v/v) and 10-20% CO_2 (v/v) (Lambert *et al.*, 1991). These changes in gas concentrations select the microbial population that will develop, the type of spoilage and the shelf life of the beef (Lambert *et al.*, 1991). The pH, in combination with film permeabaility, also controls the composition of the developing flora (Campbell *et al.*, 1979; Egan and Roberts, 1987). Low pH meats (5.4-5.6) will develop a flora consisting of only lactic acid bacteria while high pH meats (5.9-6.4) will develop a mixed flora with potent spoilage organisms present (Gill and Molin, 1991). High pH vacuum packaged meats will develop putrid off-odours and spoil rapidly (Gill and Molin, 1991).

Under vacuum storage, growth of Gram-negative bacteria is severely restricted, while Gram-positive organims dominate (Dainty and Mackey, 1992). The pseudomonads are inhibited from growing and the predominant microorganism becomes the lactic acid bacteria. *Brochothrix thermosphacta* may become a part of the spoilage flora if initial contamination levels are high (Gill and Penney, 1988). This organism seems to be more common in the spoilage flora of pork and lamb (Blickstad and Molin, 1983).

The lactic acid bacteria predominate in vacuum packaged meats for several reasons. First of all, the lactic acid bacteria are able to tolerate, reproduce and grow in very high CO_2 concentrations (100% CO_2) (Blickstad *et al.*, 1981). The lactic acid bacteria associated with vacuum packaged red meats are *Lactobacillus, Leuconostoc*, and *Carnobacterium* (Dainty and Mackey, 1992; Gill and Greer, 1992). The lactic acid bacteria also have a faster growth rate than *B. thermosphacta* and *Enterobacteriaceae* and they can inhibit the growth of competing bacteria through the

production of certain antimicrobial agents including organic acids, hydrogen peroxide, diacetyl and bacteriocins (Gill, 1982; Ahn and Stiles, 1990). The increase in shelf life of vacuum packaged meats is attributed to the combination of low O_2, the presence of CO_2 and the production of antimicrobial agents by the lactic acid bacteria (Gill, 1986; Lambert *et al.*, 1991).

The lactic acid bacteria are facultative anaerobes which are Gram-positive cocci (Gill and Greer, 1992). The lactics are usually non-motile and non-spore forming and always oxidase-negative, catalase-negative and arginine-negative. These bacteria use sugars fermentatively and produce lactic acid (Gill and Greer, 1992). The only substrates that the lactic acid bacteria can use for growth are glucose and possibly arginine, but valine and leucine can be metabolized by these bacteria to produce volatile fatty acids (acetic, isobutyric and propionic) that contribute an acid-dairy flavour to the anaerobically stored meat (Gill, 1982; Gill and Greer, 1992).

The lactic acid bacteria can have one of two fermentative pathways; homofermentative or heterofermentative. Homofermentative lactic acid bacteria produce lactic acid as the major end-product of glucose metabolism, while heterofermentative lactic acid bacteria produce a combination of carbon dioxide, ethanol and lactic acid from glucose fermentation (Gill and Greer, 1992). Pure cultures of lactic acid bacteria can contribute to an unacceptable tasting product by producing sour, acid, cheesy tastes (Egan, 1983). This type of spoilage does not occur until some time after the bacterial population reaches maximum levels (Egan, 1983).

Brochothrix thermosphacta can be found in vacuum packages and the numbers depend on a combination of pH, storage temperature, O_2 and CO_2 concentrations, packaging permeability and lactic acid concentration (Dainty and Mackey, 1991). If

low-permeability films are used, it is unlikely that *B. thermosphacta* will reach populations that could contribute to spoilage (Egan and Roberts, 1987). This organism is a Gram-positive, facultative anaerobe that is non-motile and non-spore forming (Gill and Greer, 1992). *B. thermosphacta* is a non-acid fast rod that uses sugars fermentatively, is oxidase-negative, catalase-positive and arginine-negative (Gill and Greer, 1992). This microorganism can be a part of the spoilage flora of aerobic or anaerobic environments, but has greater significance in anoxic conditions. Under aerobic conditions *B. thermosphacta* produces acetic acid and acetoin from glucose metabolism, and in anaerobic conditions lactic acid is the main end-product (Gill and Greer, 1992). Besides glucose, this organism can also metabolize valine and leucine producing strong off-odours in meat (Gill and Greer, 1992).

Under aerobic conditions, *B. thermosphacta* may not be affected by meat pH but when conditions are anaerobic pH plays a significant role in the survival of this bacteria (Dainty and Mackey, 1992). Below pH 5.8, this microorganism will not grow in anoxic conditions (Dainty *et al.*, 1983; Dainty and Mackey, 1992). The lactic acid bacteria in vacuum packaged beef slightly inhibit *B. thermosphacta's* growth (Renerre and Montel, 1986). *B. thermosphacta* is not affected by the presence of CO_2 under O_2-free storage conditions (Newton *et al.*, 1977) and it is capable of growing in atmospheres containing up to 50% CO_2 (Gardner, 1981). This facultative anaerobe is much more common on vacuum packaged lamb and pork than beef (Shaw *et al.*, 1980) due to the higher fat content of the former meats and the higher incidence of high pH beef (Egan, 1983).

Some members of the Gram-negative, facultative anaerobic *Enterobacteriaceae* (*Serratia, Hafnia* and *Enterobacter*) can also grow on vacuum-packaged meats (Kraft, 1986; Gill and Greer, 1992) and can contribute to spoilage when the pH is greater than

5.8. This group of bacteria are motile or non-motile rods that ferment sugars (glucose and then glucose-6-phosphate) usually producing gas (Gill and Greer, 1992). The *Enterobacteriaceae* can also utilize amino acids producing malodourous end products (Gill and Greer, 1992). This family of organisms is catalase-positive, oxidase-negative and can be arginine-positive or -negative (Gill and Greer, 1992).

Shewanella (Alteromonas) putrefaciens is a facultative anaerobic, Gram-negative, motile rod that is responsible for spoilage of vacuum-packaged meat due to discolouration (Nottingham, 1982). This bacterium is catalase-positive, oxidase-negative, arginine-negative and attacks sugars oxidatively (Gill and Greer, 1992). *S. putrefaciens* can be a potent spoilage organism on high pH meat (>6.0) (Egan and Roberts, 1987) both aerobically and aerobically (Lambert *et al.*, 1991). Under aerobic conditions this organism uses the amino acids cysteine and serine to produce organic sulfides. Anaerobically, this bacterium produces large amounts of hydrogen sulfide (Lambert *et al.*, 1991; Gill and Greer, 1992). If conditions favour growth of *S. putrefaciens*, it can become the dominant spoilage organism even though the bacterial numbers may be low (Gill and Greer, 1992). When anaerobic condition prevail, *A. putrefaciens* will generate H_2S, which causes meat greening due to sulfmyoglobin formation (Newton and Rigg, 1979; Gill, 1986). *A. putrefaciens* will not grow below pH 6.0, therefore this microorganism is not a problem on meat with a normal pH (Egan and Roberts, 1987).

The types of organisms recovered from 100% CO_2 are no different from those described for vacuum packaged meats (Dainty and Mackey, 1992), but 100% CO_2 does seem to produce the lowest microbial counts of all packaging atmospheres (Huffman, 1974). Storage in 100% CO_2 selectively inhibits the growth of Gram-negative spoilage bacteria on meat of normal pH and allows for the growth of lactic

acid bacteria (Brody, 1989b). The exact mechanism of CO_2 inhibition is not clear, but it may be based on interference in the microbial cell's enzyme systems by carbonic acid (H_2CO_3), which is produced from the dissolution of CO_2 into the water phase of the beef (Brody, 1989b). CO_2 inhibition could also be due to changes in the bacterial cell membrane (Sears and Eisenberg, 1961).

With 100% CO_2 there seems to be a dominance of lactic acid bacteria and they can often be the only detectable group of microorganisms (Enfors *et al.*, 1979; Blickstad and Molin, 1983). Packaging and prolonged storage in CO_2 extends the lag phase and decreases the growth rate of spoilage bacteria (Gill, 1990). *Brochothrix thermosphacta* and/or *Enterobacteriaceae* can contribute to spoilage of beef in 100% CO_2 CAP if they initially were present in the beef before packaging, meat pH is high (>6.0), or the packaging material is not completely impermeable to gases (Gill and Penney, 1985; Gill and Harrison, 1989). Some of the cheesy/dairy odour found in CAP meats can be attributed to diacetly and alcohols which can be produced by *B. thermosphacta* (Dainty and Hibbard, 1983). *B. thermosphacta,* when stored under 100% CO_2 CAP, has an increase in minimum growth temperature up to 0°C (Gill, 1990). Therefore, the effect of temperature in a CAP system is very significant (Brody, 1989b) as the growth of *B. thermosphacta* can be totally inhibited by storage in 100% CO_2 at -1.5°C (Gill and Harrison, 1989). Lower temperature seems to increase CO_2's inhibitory effects by increasing the formation of carbonic acid which can more quickly dissociate into H^+ and HCO_3- (Genigeorgis, 1985).

In the absence of other spoilage bacteria, the lactic acid bacteria can contribute to off-flavours if populations reach maximum numbers for several weeks (Egan, 1983) and these acidic fermentation products from the lactics can be of major concern (Egan and Shay, 1982; Dainty and Mackey, 1992). Homofermentative lactic acid bacteria

degrade hexoses producing strictly lactic acid as the major end-product which can contribute to sour tasting beef. Stale and rancid odours and flavours are eliminated from the CAP meat due to the absence of oxygen (Gill, 1990). If the volume of CO_2 is not sufficient to saturate the beef (Gill and Penney, 1988) or other conditions arise to promote the growth of Gram-negative microorganisms, these bacteria can thrive in the CAP beef and produce offensive off-odours and flavours (Dainty and Mackey, 1992).

d) Anoxic to Aerobic Storage

As stressed previously, it is often necessary to transfer beef from an anoxic environment to the aerobic conditions of retail display to allow the beef to "bloom" (Greer *et al.*, 1992). With the increasing interest in centralized packaging of retail ready cuts, this is becoming more important and needs careful consideration (Scholtz *et al.*, 1992a). Information is needed on the bacterial changes and spoilage development that can occur during the transition from CO_2 to air (Greer *et al.*, 1993). During this transition rapid changes in the microbial flora can occur due to changes in the film permeability and alteration of the gas atmosphere (Vanderzant *et al.*, 1982).

Previous studies have produced contradictory conclusions as to what changes occur in the bacterial population once beef stored without oxygen is transferred to air (Brody, 1989b). It has been well documented that CO_2 has a strong inhibitory effect on microbial growth (Enfors *et al.*, 1979), but this inhibition is terminated after the atmosphere is broken (the package is opened). Once placed into air the rate of microbial growth is similar to meat stored entirely under aerobic conditions (Enfors *et*

al., 1979; Banks *et al.*, 1980) but the odour and appearance of the beef may be different (Sutherland *et al.*, 1975).

Several researchers have found that prolonged vacuum or MAP storage increased the rate of deterioration of beef when transferred to aerobic conditions (Shay and Egan, 1990; Greer and Jones, 1991). Others have concluded that the inhibitory effects of CO_2 may continue to function and be expressed once the transition from CO_2 to air has occurred (Spahl *et al.*, 1981; Scholtz *et al.*, 1992a; Scholtz *et al.*, 1992b) causing a dramatic increase in shelf life (Spahl *et al.*, 1981). Pork chops that were stored in 100% CO_2 had lower bacterial counts (total anaerobes, *Pseudomonas* species and *Brochothrix thermosphacta*) on retail display than chops previously stored in MAP (25% CO_2, 75% O_2) or vacuum skin packaging (Scholtz *et al.*, 1992a). An atmosphere of 20% CO_2 and 80% N_2 before retail display produced beef steaks with lower psychrotrophic and lactic acid bacteria counts than those of comparable steaks that had been previously vacuum packaged (Christopher *et al.*, 1980).

Most of the research in this area concentrates on the transfer from vacuum to air. When vacuum packaged strip loins are transferred to simulated retail display, Gram-negative, aerobic psychrotrophs are inhibited from growing and the lactic acid bacteria are a dominant part of the flora of all steaks displayed for 5-30 days (Vanderzant *et al.*, 1982). Following vacuum storage, the number of recoverable pseudomonads decreases and the number of lactics increase during subsequent aerobic conditions (Newsome *et al.*, 1984). An interesting study was done with aerobic storage before vacuum and its effects on microbial growth of beef (Foegeding *et al.*, 1983). Initially, *Pseudomonas* species dominated but numbers decreased as storage time under vacuum increased. *B. thermosphacta* were also detectable and their populations were low and seemed to decrease very quickly with storage time (Foegeding *et al.*, 1983). After 14

days the lactic acid bacteria completely dominated the flora due to the lack of oxygen in the vacuum package and this group of microorganisms appears to produce an inhibitor to *B. thermosphacta* (Roth and Clark, 1975).

The lactic acid bacteria appear to dominate the flora of beef once the beef is transferred to aerobic conditions from atmospheres of high CO_2. Lactic acid bacteria continue to dominate the flora of pork that were previously stored in MAP (Enfors *et al.*, 1979), and CAP (Scholtz *et al.*, 1991a; Greer *et al.*, 1993) and then transferred to air. As CO_2 storage time increased there was a significant increase in the numbers of lactics present on the meat placed into retail conditions. A 1.5 log cycle increase in lactic acid bacteria was found for each 3 week CO_2 storage interval (Greer *et al.*, 1993). The pseudomonads began to emerge after a 43 hour lag phase but they never outgrew the lactics (Greer *et al.*, 1993). The enterics and *B. thermosphacta* did grow after a time but their populations were low in numbers (Greer *et al.*, 1993). However, once placed onto retail display after CAP storage, the enterics, *B. thermosphacta* and pseudomonads were not detected at any storage time interval (Greer *et al.*, 1993). It may be that the lactic acid bacteria can suppress the aerobic spoilage bacteria even under aerobic retail display conditions (Greer *et al.*, 1992).

e) Pathogens

The lactic acid bacteria are also important to the safety of vacuum packaged beef by inhibiting the growth of pathogens (Egan, 1983). Pathogens are inhibited because the lactics produce acid, which lowers the pH. Lactics also produce bacteriocins, diacetyl and hydrogen peroxide (Genigeorgis, 1976) all of which inhibit some pathogens. Decreasing the pH creates a situation where higher growth temperatures

are required for pathogens such as *Clostridium botulinum, Clostridium perfringens* and salmonellae (Kraft, 1986).

An inhibitory effect is found with pathogens which is similar to that of spoilage organisms. CAP packaging in 100% CO_2 extends the lag phase, decreases the growth rate and increases minimum growth temperature (Gill, 1990). Storage in CO_2 at temperatures below 5.0°C completely inhibits the growth of some pathogens (*Aeromonas hydrophila, Yersinia enterocolitica, Listeria monocytogenes, Salmonella, Escherichia coli, Staphylococcus aureus* and salmonellae) on meat of normal pH (Nottingham, 1982; Brody, 1989b; Gill, 1990).

Unfortunately, the precise mechanisms that permit certain microorganisms to grow more rapidly under certain storage conditions and at lower storage temperatures is still not understood nor is the difference in CO_2-tolerance for different bacteria (Dainty and Mackey, 1992). The answers to these questions will help us to control microbial growth more effectively and decrease spoilage (Dainty and Mackey, 1992).

Statement of the Problem

Fresh beef can be stored for extended periods of time under 100% CO_2. Unfortunately, once the meat is transferred to the aerobic conditions of retail display, quality deteriorates rapidly. There is a significant increase in surface discolouration and off odour formation, while retail acceptability and case life decrease. The objective of the research was to evaluate the changes in colour, shelf life, consumer acceptability and bacterial quality and quantity of retail ready beef steaks on aerobic retail display after extended storage in 100% CO_2. Then using statistically valid methods, evaluate the changes in colour, shelf life, bacterial quality and bacterial quantity in terms of consumer acceptability. Answers to these questions should provide insights into the optimal storage times and temperatures for both CO_2 storage and subequent retail display.

Materials and Methods

Acceptability of Different Colour Stability Muscles After Storage in 100% CO_2

Three beef muscle types which were chosen because of differences in colour stability. Grade A1 *longissimus dorsi* (good colour stability), *psoas major* (poor colour stability) and *semimembranosus* muscles (intermediate colour stability) (O'Keefe and Hood 1980-81a; Ledward, 1985) were obtained from a federally-inspected abattoir (Cargill Foods, High River, Alberta, Canada). The muscles were vacuum-packaged and stored at 0°C for 24 hours prior to being boxed, packed in dry ice and transported under commercial conditions to he Agriculture and Agri-Food Canada, Lacombe Research Station, Lacombe, Alberta, Canada. Delphi temperature loggers (Delphi Industries Limited, Auckland, New Zealand), randomly placed throughout the boxes, measured the average temperature during transport. The average temperature was 1.71°C.

Upon arrival, the muscles were removed from the vacuum package and 2.5 cm of meat was trimmed from each end of the muscle. Then the muscles were trimmed of subcutaneous fat and cut into 2.5 cm thick steaks. Average steak dimensions were 14.5cm x 7.5 cm for *longissimus dorsi*, 11.5 x 9.0 cm for *psoas major*, and 12.5 x 8.0 cm for *semimembranosus*. A total of 54 steaks (18 steaks from each of the three muscles) were randomly assigned to a controlled atmosphere package (CAP) in 100% CO_2.

All steaks were packaged in foil-laminate pouches (60 gauge biax nylon, 60 gauge co-extrusion of low density polyethylene and ethylene acrylic acid, 35 gauge aluminum

foil, 75 gauge EAA and 300 gauge linear low density polyethylene film; American National Can Co., Neenah, WI, U.S.A.), having a gas transmission rate 0f <0.01 $cc/m^2/24$ hours. Each package contained 6 steaks (all 3 muscles, 2 steaks of each muscle). Packaging was done in a Captron III packaging system (RMF, Grandview, MO U.S.A.). CAP was acheived by back-flushing the foil-laminate pouch with 2.5 L CO_2/kg of meat. After packaging all CA packages had residual oxygen levels <300 ppm, as measured by a Mocon Oxygen Headspace Analyzer (model number HS 750 Mocon Modern Controls Inc., Minneapolis, MN, U.S.A.).

Steak packages were randomly assigned to the storage temperature of -1.5°C (+/- 0.2°C), which represents the optimal storage temperature for fresh beef, and to one of nine storage intervals (0, 3, 6, 9, 12, 15, 18, 21, or 24 weeks).

After each storage interval, residual oxygen levels were measured on each CAP package. Steaks were removed from the foil-laminate pouch and placed onto individual styrofoam trays (Scott National, Calgary, Alberta, Canada). Odour was evaluated on each steak by a trained 5 member sensory panel. Odour was evaluated using a 4-point descriptive scale of 1 (no off odour) to 4 (prevalent off odour). Surface pH measurements were taken (three measurements per steak, each at a different location) using a Horiba pH meter, (model number F-12 Horiba, Ltd., Kyoto, Japan), with a flat surface gel-filled combination electrode (model number 91-36, ORION Research Incorporated Laboratory Products Group, Boston, MA, U.S.A.)

After the above measurements, the steaks were overwrapped in oxygen-permeable polyvinyl chloride film (Vitafilm Choice Wrap, Goodyear Canada Inc., Toronto, Ontario, Canada) having an oxygen transmission rate of 8000 $cc/m^2/24$ hours, and placed in a fan-circulated, horizontal-type retail case (Hill Refrigeration of Canada Ltd., Barrie, Ontario, Canada). To simulate retail conditions, the temperature of the

display case was 6.3°C and the illumination was incandescent lighting 12 hours/day to give an intensity of 750 lux at the steak surface.

When all of the steaks had been placed on retail display colour was evaluated by both sensory and objective methods. A 5 member sensory panel evaluated colour, % discolouration and retail acceptance. Colour intensity was evaluated using a 9-point descriptive scale of 0 (completely discoloured), 1 (white), 4 (pale red) and 8 (extremely dark red). Surface discolouration was evaluated using a 7-point descriptive scale of 1 (0%) to 7 (100%) and retail acceptance was evaluated using a 7-point hedonic scale of 1 (extremely undesirable) to 7 (extremely desirable). Objective colour evaluation was performed using a Macbeth spectrophotometer (Prism Instruments, Whitby, Ontario; Canada). Three colour readings were taken to provide reflectance values from 360-740 nm. Linear interpolation produced the desired wavelengths of 474 nm, 525 nm, 572 nm and 610 nm. These wavelengths were used in equations based on standards that represented 100% of each pigment. CIE L*a*b* values (L* is the light-dark axis, a* is the red-green axis and b* is the yellow-blue axis), as well as the quantitation of muscle pigments (deoxymyoglobin, metmyoglobin and oxymyoglobin), were determined according to the methodology described in Guidelines for Meat Colour Evaluation (AMSA, 1991).

Colour, by both sensory and objective measurements, was evaluated 0, 1, 2, 4, 6, 24, and 30 hours after removal from CO_2 storage and re-wrapping. After 30 hours, the oxygen-permeable film was removed and odour evaluation and surface pH measurements were again taken. All steaks were wrapped in Multivac barrier bags (Vac #3R, 8 x 12, Unisource Canada, Inc., Edmonton, Alberta, Canada), vacuum-packaged and frozen at -30°C for organoleptic evaluation.

Steaks were cooked in a convection oven (Bakers Pride Canada, Model X700, Lachine, Quebec, Canada) to an internal temperature of 75°C. The meat was cut into 1.5 cm^2 cubes. Palatability evaluation was evaluated by a 7 member trained and experienced standard laboratory panel. Steak characteristics that were evaluated were flavour desirability, off-flavour intensity and off-flavour description. Flavour description was evaluated using a 9-point hedonic scale of 1 (extremely undesirable) to 9 (extremely desirable) and off-flavour intensity was evaluated using a 4-point descriptive scale of 1 (prevalent off-flavour) to 4 (no off-flavour). Off-flavour description was evaluated by panel members in their own words describing any off-flavours found.

Microbiological Quality of Retail Beef Steaks as a Result of Storage in CO_2

This work comprised two separate but comparable studies. For each study, grade A1 *longissimus dorsi* muscles were obtained from Cargill Foods, vacuum packaged and shipped under commercial conditions at 0-2°C as described previously. The muscles were trimmed free of visible subcutaneous fat and 2.5 cm of meat was trimmed off of each end. The muscles were cut into 300, 2.5 cm thick steaks, 14.5cm x 7.5 cm.

Steaks were randomly placed on plastic (polypropylene) cafeteria trays, 6 steaks/tray, and placed inside oxygen-impermeable "EVOH" bags (60 gauge ethyl vinyl alcohol co-polymer, 3 mil polyethylene; Packaging Industries, Inc., San Leandro, CA U.S.A.), having a gas transmission rate of 0.0003 $cc/m^2/24$ hours. Each bag was filled with 2.5 L 100% CO_2/kg of meat.

Packages were randomly placed inside cardboard boxes containing Delphi temperature loggers and randomly designated to one of five storage intervals (0, 6, 12, 18, or 24 weeks) at one of two storage temperatures. The two temperatures (-1.5°C +/- 0.2°C and 2.0°C +/- 0.2°C) were chosen to represent ideal and commercial storage conditions, respectively. One box of 5 packages each was assigned to each storage interval at each temperature.

Following each storage interval steaks were removed from the CO_2 master-pack, placed on individual styrofoam trays (Scott National, Calgary, Alberta, Canada) and overwrapped with the oxygen-permeable polyvinyl chloride film having an oxygen transmission rate of 8000 cc/m^2/24 hours. Retail steaks were randomly placed in a fan-circulated, horizontal-type retail case (Hill Refrigeration of Canada Ltd., Barrie, Ontario, Canada) as described previously.

The mean surface temperature of the steaks during the display period was 6.3°C, which represents commercial temperature conditions (part 1 of this experiment). Part 2 of the experiment was identical except for the fact that the retail temperature had been lowered to 2.6°C to reflect commercially attainable conditions. All methods were identical in part 2 except for the fact that the steaks were only stored at 2.0°C, rather than at both -1.5°C and 2.0°C, and the storage interval only went to 18 weeks, rather than 24 weeks. Part 2 had a total of 120 steaks rather than 300.

Five steaks were evaluated by an experienced 5 member sensory panel on days 0, 2, 4, 6, 8, and 10 of retail display at each storage interval (0, 6, 12, 18, and 24 weeks) for colour intensity, surface discolouration, retail acceptance, off-odour intensity, and odour acceptability. Colour was evaluated using a 8-point descriptive scale of 0 (completely discoloured), 1 (white), 4 (pale red) and 8 (extremely dark red). Surface discolouration was evaluated using a 7-point descriptive scale of 1 (0%) and 7

(100%). Retail acceptance was evaluated using a 7-point hedonic scale of 1 (extremely undesirable) and 7 (extremely desirable). Off-odour intensity was evaluated using a 4-point descriptive scale of 1 (no off-odour and 4 (prevalent off-odour) and odour acceptability was evaluated using a 5-point descriptive scale of 1 (acceptable) and 5 (unacceptable). Retail case life was quantified by assuming that rejection would occur at the time in days that scores for retail appearance and odour acceptance declined to 3.5 (Greer and Murray, 1991).

Steaks were sampled by the aseptic removal of $10cm^2$ of tissue from the surface at one location only. The sample was combined with 90 ml of 0.1% peptone-water and homogenized for 2 minutes using a Stomacher Lab Blender (Model 400, Seward Medical, London, UK). After sensory evaluation and bacterial sampling all steaks were discarded. A total of 10 steaks (5 from each storage temperature) were sampled at each display time for part 1 and 5 steaks for part 2.

Following serial, ten-fold dilutions in 0.1% peptone-water, lactic acid bacteria, *Brochothrix thermosphacta*, pseudomonads and total bacterial count were determined by the spread plate technique and *Enterobacteriaceae* (enterics) by the pour plate technique.

Enterics were determined after incubation for 18-24 hours at 35°C using overlaid plates of violet red bile glucose agar (VRBGA) (Difco). Total counts were determined using plate count agar (PCA) (Difco) after 10 days incubation at 2.0°C. Pseudomonads were determined using cephaloridine-fucidin-cetrimide agar (CFC) (Mead and Adams, 1977) after 2 days incubation for 3 days at 25°C. *B. thermosphacta* were enumerated on streptomycin sulfate-thallous acetate-actidione agar (STAA) (Gardner,1966) after incubation for 3 days at 25°C. Lactic acid bacteria were determined on MRS agar (Difco) after anaerobic incubation for 3 days at 25°C

using a BBL anaerobic system containing 5-10% CO_2 (Becton and Dickenson Co., Cockeysville, MD U.S.A.). The selective properties of the above media have been determined (Baird *et al.*, 1987).

Statistical Analysis

The main variables considered in this study were storage time, replicate and display hour. Data for colour, discolouration, retail acceptance, pH, odour, L*a*b* values and meat colour pigment percentages were analyzed two factors at a time using a general linear model (SAS, version 6.7, 1989). This gave an analysis of variance with a comparison of least squares means based on the least significant difference with one degree of freedom.

The main effects considered in the microbiological portion of this study were storage temperature, storage time, retail storage temperature, days on retail display and replicate. All statistical analysis for microbiological data includes five replicates at each of the two storage temperatures. Statistically significant differences were determined following analysis of variance (SAS, 1989) which gave an analysis of variance with a comparison of least squares means based on the least significant difference with one degree of freedom.

The significance of differences between means was determined using the Students's 't' test.

Results

Acceptability of Different Colour Stability Muscles After Storage in 100% CO_2

Sensory Colour Evaluation

Table 1 a, b and c shows the summary of the mean sensory panel colour scores for *longissimus dorsi, semimembranosus* and *psoas major* steaks stored at -1.5°C in 100% CO_2. Colour intensity scores were 0 (completely discoloured), 1 (white), 2 (pale pink), 3 (pink), 4 (pale red), 5 (bright cherry red), 6 (slightly dark red), 7 (moderately dark red) and 8 (extremely dark red). Colour means increased for *longissimus dorsi* steaks as storage time and retail display hour both increased. All steaks were bright cherry red in colour. *Semimembranosus* colour means also increased with increases in storage time (weeks) and display hour. *Psoas major* steaks were slightly dark red at 24 and 30 hours of display for 0 and 3 week samples. During the period of 6 to 24 weeks anoxic storage, *psoas major steaks* had mean colour increases.

Sensory Surface Discolouration

Table 2 a, b and c shows the sensory panel percent surface discolouration mean scores for the three muscle types. The mean scores ranged from 1 to 7 with the following meanings: 1 (0% discolouration), 2 (1-10%), 3 (11-25 %), 4 (26-50%),

Table 1a. Sensory colour means for *longissimus dorsi* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	5.48[1]	5.24	5.37	5.12	5.02	5.21	5.35	5.44	5.50
1	5.53	5.13	5.22	5.06	5.19	5.13	5.40	5.44	5.79
2	5.48	5.14	5.08	5.20	5.40	5.21	5.44	5.31	5.73
4	5.52	5.11	5.30	5.36	5.38	5.29	5.47	5.42	5.83
6	5.45	5.15	5.35	5.08	5.24	5.49	5.64	5.51	5.96
24	5.48	5.49	5.47	5.13	5.36	5.61	5.51	5.55	5.92
30	5.48	5.57	5.55	5.35	5.24	5.64	5.59	5.31	5.85
S.E.	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12

Table 1b. Sensory colour means for *semimembranosus* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	5.66[1]	5.74	5.65	5.78	5.59	5.23	5.71	5.65	5.55
1	5.73	5.68	5.57	5.51	5.54	5.27	5.78	5.81	5.73
2	5.80	5.69	5.52	5.55	5.71	5.37	5.68	5.83	5.83
4	5.85	5.63	5.55	5.68	5.85	5.24	5.82	5.92	5.93
6	5.77	5.52	5.56	5.67	5.73	5.51	5.85	6.10	5.88
24	5.94	5.83	5.77	5.80	5.88	5.59	5.59	5.70	6.04
30	5.95	5.89	6.02	5.76	5.77	6.06	5.55	5.45	5.92
S.E.	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.18	0.17

Table 1c. Sensory colour means for *psoas major* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	5.65[1]	5.45	5.51	5.47	5.36	5.25	5.79	5.26	5.60
1	5.73	5.27	5.45	5.54	5.46	5.31	5.85	5.44	5.77
2	5.75	5.23	5.32	5.46	5.77	5.27	5.79	5.68	5.92
4	5.82	5.46	5.38	5.61	5.75	5.31	6.34	5.31	6.00
6	5.85	5.34	5.57	5.59	5.75	5.58	6.32	5.18	5.89
24	6.08	6.11	5.68	5.73	5.92	5.22	5.58	5.56	5.83
30	6.17	6.11	5.67	5.47	5.85	5.08	5.62	5.34	5.69
S.E.	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.17	0.14

[1]Colour scores based on a 9-point descriptive scale (0=completely discoloured , 1=white and 8= extremely dark red).

Table 2a. Sensory percent discolouration means for *longissimus dorsi* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	1.23[1]	1.03	1.02	1.00	1.08	1.42	1.78	1.35	1.48
1	1.22	1.02	1.13	1.05	1.27	1.63	2.17	2.54	1.81
2	1.43	1.00	1.15	1.05	1.48	2.13	2.70	2.71	1.98
4	1.15	1.03	1.18	1.07	2.02	2.56	2.78	2.90	2.27
6	1.13	1.08	1.25	1.40	2.48	3.04	3.02	3.27	2.41
24	1.45	2.14	2.15	2.34	3.23	4.56	3.80	4.63	3.81
30	1.57	2.58	2.83	3.02	3.46	5.13	4.38	4.88	4.27
S.E.	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29

Table 2b. Sensory percent discolouration means for *semimembranosus* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	1.01[1]	1.37	1.23	1.15	1.20	1.19	2.44	1.21	1.95
1	1.12	1.50	1.43	1.28	1.35	1.56	2.78	2.00	2.38
2	1.36	1.23	1.48	1.37	1.73	2.00	3.28	2.56	2.54
4	1.41	1.28	1.52	1.38	2.06	2.63	3.88	2.88	3.15
6	1.42	1.23	1.72	1.52	2.38	2.68	4.13	3.54	3.60
24	1.49	2.12	2.30	2.47	3.50	4.69	5.40	5.35	5.13
30	1.63	2.06	2.68	3.31	3.79	4.79	5.68	5.71	5.33
S.E.	0.30	0.30	0.30	0.30	0.30	0.29	0.30	0.30	0.30

Table 2c. Sensory percent discolouration means for *psoas major* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	1.23[1]	1.13	1.10	1.00	1.38	1.83	3.89	3.08	1.93
1	1.46	1.20	1.13	1.30	2.13	2.31	4.30	4.06	2.88
2	1.55	1.10	1.25	1.40	2.33	3.10	4.75	4.48	3.15
4	1.65	1.37	1.68	1.67	3.00	3.67	4.92	4.85	3.71
6	1.80	1.47	1.70	1.95	3.33	4.03	5.32	5.15	4.23
24	2.45	3.63	3.03	3.64	4.38	5.40	5.71	5.85	5.73
30	2.78	3.79	3.57	4.04	4.77	5.75	5.93	5.92	5.69
S.E.	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32

[1]Percent discolouration scores based on a 7-point descriptive scale (1=0% surface discolouration and 7=100% surface discolouration).

5 (51-75%), 6 (76-99%) and 7 (100%) surface discolouration. Surface discolouration means increased significantly with increasing storage time and display hour for *longissimus dorsi* steaks ($P<0.05$). All *longissimus dorsi* steaks had less than 50% surface discolouration throughout the 24 week interval. Mean surface discolouration values also increased significantly with increases in anoxic storage and retail display hour for *semimembranosus* steaks ($P<0.05$). Steaks were scored as having greater than 50% surface discolouration at 18 weeks of CO_2 storage. *Psoas major* steaks followed a similar trend with significant increases in surface discolouration means as storage time and hours on display both increased ($P<0.05$). The *psoas major* steaks discoloured the quickest by having greater than 50% surface discolouration at 15 weeks.

Sensory Retail Acceptance

Table 3 a, b and c summarizes the ANOVA for sensory retail acceptance mean scores for the three beef steaks. The range of mean scores was 1 (extremely undesirable), 2 (undesirable), 3 (slightly undesirable), 4 (neither desirable nor undesirable), 5 (slightly desirable), 6 (desirable) and 7 (extremely desirable). All steak means decreased significantly as storage time and hours on display both increased ($P<0.05$). *Longissimus dorsi* steaks did not become undesirable until 15 weeks (24 hours). *Semimembranosus* steaks became undesirable at 12 weeks (30 hours) and *psoas major* steaks were undesirable at 9 weeks (30 hours).

Table 3a. Sensory retail acceptance means[1] for *longissimus dorsi* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	5.83[2]	5.93	6.10	5.98	5.60	5.38	4.79	5.21	5.12
1	5.80	6.20	5.85	5.71	4.94	4.96	4.45	4.54	4.71
2	5.68	6.28	5.65	5.56	4.46	4.67	4.38	4.17	4.50
4	5.78	6.08	5.48	5.40	4.13	4.21	4.22	3.96	4.06
6	5.87	5.94	5.52	5.08	3.90	3.79	3.91	3.52	4.04
24	5.68	4.75	4.28	3.91	3.38	2.56	2.68	2.29	2.79
30	5.60	4.33	3.57	3.46	3.06	2.23	2.36	1.94	2.58
S.E.	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

Table 3b. Sensory retail acceptance means for *semimembranosus* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	5.35	5.53	5.80	5.40	5.54	4.96	4.28	5.58	4.67
1	5.48	5.67	5.73	4.97	5.21	4.79	3.89	4.94	4.19
2	5.46	5.66	5.50	4.98	4.73	4.27	3.53	4.13	3.98
4	5.43	5.68	5.20	4.82	4.04	3.85	3.08	3.67	3.69
6	5.38	5.51	5.18	4.50	3.73	3.71	2.80	3.31	3.44
24	5.38	4.51	4.02	3.86	3.21	2.52	1.78	1.65	2.06
30	5.35	4.57	3.55	3.08	2.75	2.19	1.52	1.50	2.00
S.E.	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28

Table 3c. Sensory retail acceptance means for *psoas major* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	5.43[2]	5.51	5.83	5.77	5.38	4.83	3.39	4.04	4.43
1	5.35	5.48	5.72	5.43	4.40	4.50	2.75	3.63	3.50
2	5.33	5.34	5.65	4.98	4.40	4.00	2.61	3.00	3.42
4	5.32	5.28	5.00	4.49	4.08	3.29	2.27	2.46	3.08
6	5.29	4.96	4.78	4.31	3.35	3.06	2.14	2.38	2.71
24	5.28	3.29	3.52	3.35	3.38	2.15	1.69	1.73	1.81
30	5.22	3.32	3.45	2.98	2.83	1.88	1.58	1.56	1.67
S.E.	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26

[1]Means based on a 7-point hedonic scale (1=extremely undesirable and 7=extremely desirable).
[2]Means less than 4.0 are no longer desirable.

Sensory Off Odour Intensity

The summary for off odour intensity for all beef steaks is shown in Table 4 a, b and c. Means were 1 (no off odour), 2 (slight off odour), 3 (moderate off odour) and 4 (prevalent off odour). Off odour increases significantly with increases in display hour and storage time for the three muscles types ($P<0.05$). At no time did any retail display hour/storage time combination cause any steaks from any of the muscles to develop prevalent off odours. *Longissimus dorsi* and *semimembranosus* steaks developed slight off odours at 3 weeks storage and *psoas major* steaks developed slight off-odours at 9 weeks.

Surface pH

Table 5 shows the summary for the surface pH measurements at different storage times for all three steaks. The pH decreased significantly ($P<0.05$) for all muscle types as storage time in CO_2 increased. The *psoas major* steaks had the greatest decrease in mean pH values with time (5.71 to 5.06). *Longissimus dorsi* and *semimembranosus* steaks seemed to be more pH stable with pH means only decreasing to 5.19 and 5.24 respectively.

ANOVA of mean surface pH measurements illustrating the effects of storage time (weeks) and display hour is also shown in Table 5. The pH measurements taken at 0 hours show that as storage time increases from 3 to 24 weeks, there is a gradual decrease in pH means and these differences are significant especially when comparing means early in storage (3 and 6 weeks) to pH means at the end of the storage time (21 and 24 weeks). When comparing display hour no significant differences are found

Table 4a. Sensory off odour intensity means for *longissimus dorsi* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	1.03[1]	1.55	1.38	1.56	1.80	1.69	2.05	2.17	2.52
30	1.12	2.02	2.28	2.28	2.56	2.82	2.99	2.94	3.31
S.E.	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12

Table 4b. Sensory off odour intensity means for *semimembranosus* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	1.08[1]	1.47	1.53	1.90	1.78	1.85	2.23	1.97	2.77
30	1.38	2.03	2.13	2.44	2.48	2.71	3.29	3.08	3.46
S.E.	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13

Table 4c. Sensory off odour intensity means for *psoas major* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	1.05[1]	1.50	1.30	1.66	1.92	1.85	2.50	2.34	2.50
30	1.43	1.67	1.89	2.01	2.25	2.83	3.05	2.83	3.06
S.E.	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10

[1]Off odour intensity scores based on a 4-point descriptive scale (1=no off odour and 4=prevelant off odour).

Table 5. ANOVA of surface pH measurements of beef steaks illustrating the effect of display hour.

Storage Time (Weeks)	Display Hour	*Longissimus dorsi*	*Semimembranosus*	*Psoas major*
0	0	5.66[1]a	5.66 b	5.71 a
3	0	5.49 bc	5.59 bc	5.50 bc
6	0	5.50 bc	5.64 b	5.50 bc
9	0	5.29 ef	5.65 b	5.31 c
12	0	5.19 g	5.27 f	5.20 de
15	0	5.33 de	5.41 d	5.27 cd
18	0	5.27 efg	5.27 f	5.10 f
21	0	5.22 fg	5.29 f	5.13 ef
24	0	5.25 efg	5.37 e	5.42 bc
0	30	5.35 de	5.37 e	5.52 b
3	30	5.57 b	5.62 b	5.50 bc
6	30	5.45 c	5.62 b	5.31 c
9	30	5.29 ef	5.71 a	5.27 cd
12	30	5.26 efg	5.39 e	5.25 cd
15	30	5.28 efg	5.38 e	5.09 f
18	30	5.27 efg	5.24 f	5.09 f
21	30	5.23 fg	5.30 ef	5.07 f
24	30	5.42 cd	5.55 c	5.27 ef
		SE=0.03	SE=0.07	SE=0.03

[1]Means in the same column bearing a common letter are not significantly different (P>0.05).

between pH means taken at 0 hour and 30 hours for any storage time week for *psoas major* steaks ($P>0.05$). *Longissimus dorsi* steaks show display hour pH means that are significantly different from each other at 0 and 24 weeks ($P<0.05$).

L* a* b* Values

Table 6 a, b and c shows the summary for L* mean values for the three types of muscles. L* means increase slightly as both storage time (weeks) and time on display (hours) increase for *longissimus dorsi* steaks. *Semimembranosus* and *psoas major* steaks have significantly different L* means scores and also increase with increasing storage time and display hour.

Table 7 a, b and c shows the summary for a* mean scores for the three different muscles. All steaks show significant decreases in a* means (steaks are less red) as storage time increases from 0 to 24 weeks. Mean a* values increase from 0 to 4 hours of retail display and then become less red (a* means decrease) from 6 to 30 hours of display.

The summary for the mean b* scores is shown in Table 8 a, b and c. These means decrease with extended anoxic storage most significantly at 21 and 24 weeks. *Psoas major* steaks had significantly lower b* values compared to the other two muscle types.

Table 6a. L*[1] means for *longissimus dorsi* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	46.83	48.41	49.34	51.85	49.53	50.25	51.71	52.93	51.85
1	48.70	49.99	51.98	52.41	50.83	50.49	52.55	53.30	51.66
2	49.31	50.14	51.32	52.90	50.40	50.19	48.23	50.76	52.56
4	49.68	51.27	51.78	52.33	50.95	50.41	44.08	49.84	52.37
6	49.52	51.14	52.03	53.00	50.98	50.00	52.03	53.26	52.41
24	49.28	50.41	51.65	52.20	51.48	50.54	52.58	52.55	51.97
30	50.29	51.31	51.12	52.86	51.61	48.74	52.67	52.71	51.93
S.E.	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11

Table 6b. L* means for *semimembranosus* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	46.17	46.42	48.91	50.05	46.54	47.75	45.95	49.23	48.08
1	47.38	48.66	50.51	50.54	47.47	50.15	50.75	50.11	48.80
2	49.72	48.24	50.97	51.33	47.39	49.85	46.99	50.08	49.16
4	49.35	49.84	51.40	51.24	47.93	49.58	46.59	48.25	48.84
6	49.48	49.01	51.42	51.49	45.35	48.65	50.38	50.01	49.16
24	48.46	49.40	50.15	51.52	48.27	49.26	51.13	49.41	50.14
30	49.87	50.23	50.29	51.49	48.73	49.82	50.92	49.36	47.48
S.E.	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Table 6c. L* means for *psoas major* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	44.76	47.47	45.14	49.31	49.14	47.80	49.03	50.95	49.20
1	47.43	49.41	50.68	50.18	49.58	49.04	50.50	51.45	49.34
2	47.14	48.97	50.48	50.54	50.01	49.2	45.88	50.66	49.15
4	48.33	49.81	50.57	50.34	49.92	49.89	46.61	50.98	48.83
6	47.72	49.02	50.74	49.54	49.88	46.18	49.41	49.72	49.66
24	49.05	49.38	49.83	50.28	49.61	49.57	49.43	46.55	49.62
30	48.62	50.06	43.78	50.39	49.75	49.09	50.89	50.81	49.62
S.E.	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

[1]"L" is a measurement of the lightness and darkness of the sample, the larger the value the lighter it is.

Table 7a. a*[1] means for *longissimus dorsi* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	13.98	14.53	12.07	12.03	14.87	14.40	13.36	13.13	14.34
1	16.50	19.22	17.06	15.25	17.94	17.24	16.28	15.58	17.45
2	16.44	19.80	17.95	16.15	18.57	18.21	16.42	16.34	16.29
4	16.87	19.91	18.26	16.05	18.53	17.66	15.43	18.45	16.51
6	17.87	19.67	18.02	15.70	18.35	17.89	15.39	15.03	15.63
24	19.52	19.41	17.28	14.97	16.73	15.72	13.30	13.22	13.62
30	19.18	19.13	16.87	14.26	16.13	13.43	12.71	12.52	11.48
S.E.	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13

Table 7b. a* means for *semimembranosus* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	13.64	15.83	12.91	15.50	16.60	16.93	19.23	16.00	17.07
1	16.63	19.44	18.52	19.11	19.40	17.81	19.22	17.13	17.22
2	15.45	20.19	19.27	19.12	19.60	18.69	18.25	16.97	17.76
4	16.63	19.53	19.30	18.98	17.17	18.23	17.89	16.08	16.14
6	16.95	19.17	19.20	18.59	18.64	18.42	16.21	15.03	15.48
24	18.91	18.97	17.72	16.50	15.43	15.72	12.33	11.78	12.48
30	17.95	18.38	17.39	15.97	16.35	12.00	11.87	11.31	11.80
S.E.	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Table 7c. a* means for *psoas major* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	14.41	17.38	18.35	14.33	15.19	15.50	16.40	16.05	16.18
1	16.66	19.09	18.44	17.72	18.25	17.87	17.48	17.03	16.11
2	16.62	19.25	18.02	17.56	17.70	17.57	16.14	16.78	15.13
4	15.91	17.92	17.34	17.50	17.00	16.43	14.76	15.10	13.17
6	16.41	17.81	17.00	17.57	16.20	14.78	13.56	14.76	12.23
24	17.63	15.68	13.76	14.10	12.82	12.12	9.40	14.07	8.77
30	16.15	14.69	15.81	13.26	12.43	13.20	9.10	10.43	9.82
S.E.	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13

[1]"a" is a measurement of the redness and greenness of the sample, the larger the value the more red it is.

Table 8a. b*[1] means for *longissimus dorsi* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	6.94	6.97	6.06	6.24	7.60	7.27	6.69	6.76	7.41
1	8.56	10.47	9.35	8.37	9.94	9.33	8.79	8.37	9.86
2	8.44	10.66	9.62	9.06	10.28	10.00	8.69	9.02	9.11
4	8.60	10.52	9.82	8.74	10.23	9.71	8.29	9.17	9.58
6	9.37	10.63	9.73	8.56	10.34	10.00	8.46	8.32	9.13
24	10.27	10.51	9.38	8.41	9.55	8.90	7.64	7.94	8.50
30	9.95	10.19	9.28	7.99	9.21	8.58	7.43	7.65	8.11
S.E.	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06

Table 8b. b* means for *semimembranosus* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	7.89	7.55	6.41	8.35	8.47	9.03	9.34	8.15	8.85
1	9.42	10.39	10.32	10.61	10.52	9.95	11.02	9.37	9.96
2	8.62	10.66	10.79	10.49	10.65	10.51	10.46	9.33	10.00
4	9.42	10.13	10.74	10.49	10.43	10.27	10.16	9.15	9.33
6	9.72	10.22	10.65	10.23	12.25	10.35	9.63	8.68	9.12
24	10.81	10.44	10.06	9.53	8.80	9.33	8.51	7.89	7.85
30	10.23	9.57	10.20	9.37	9.17	8.81	8.52	8.10	7.74
S.E.	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13

Table 8c. b* means for *psoas major* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	7.06	9.03	8.12	6.87	7.62	7.71	8.51	8.36	8.32
1	8.95	10.31	9.94	9.27	9.94	9.58	9.72	9.56	9.25
2	8.92	10.39	9.53	9.09	9.55	9.49	9.20	9.71	9.02
4	8.41	9.45	9.26	9.14	9.43	9.26	8.74	9.12	8.51
6	8.80	9.74	9.27	9.43	9.13	11.91	8.33	9.38	8.09
24	9.88	9.17	8.01	7.74	9.18	7.49	7.12	8.67	7.66
30	8.95	8.45	11.19	7.58	7.89	8.30	7.46	7.81	7.47
S.E.	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07

[1]"b" is a measurement of the yellowness and blueness of the sample, the larger the value the more yellow it is.

Pigment Percentages

Table 9 a, b and c shows the summary for percent metmyoglobin means. At any one storage time/display hour mean score, the percent metmyoglobin is significantly lower for the *longissimus dorsi* steaks compared to *semimembranosus* and *psoas major* steaks. For all three muscle types, the percent metmyoglobin increases with increasing time in storage and time on retail display.

The percent deoxymyoglobin summary is shown in Table 10 a, b and c. All three muscle types had significant amounts of deoxymyoglobin present at 0 hours of display (immediately after transfer from anoxic storage) for all storage intervals (0 to 24 weeks). During retail display of 1 to 30 hours deoxymyoglobin was not detected in any of the steaks sampled at 3 to 24 weeks. Deoxymyoglobin was detected in small amounts (less than 1%) in *psoas major* steaks at 0 weeks. Significant differences in mean scores were found for the different storage weeks but no consistent trends were found.

Table 11 a, b and c shows the effects of storage time and hours of retail display on percent oxymyoglobin means. All three muscles had significant increases in percent oxymyoglobin as hours of retail display increased from 0 to 6 hours at weeks 3 to 24 of anoxic storage. The amount of oxymyoglobin decreased significantly after 6 hours of display for all muscle types. Overall, *longissimus dorsi* steaks had higher percentages of oxymyoglobin than *semimembranosus* and *psoas major* steaks. *Psoas major* steaks had significantly lower mean oxymyoglobin percentages when compared to the other two muscle types at any storage time/hour of display interval.

Table 9a. Percent metmyoglobin[1] means for *longissimus dorsi* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	3.20	0.00	5.44	2.57	0.00	1.65	1.95	3.58	2.79
1	3.42	0.00	0.32	1.32	0.00	1.19	1.28	1.82	2.20
2	5.15	0.00	0.03	0.81	0.56	2.11	2.15	1.45	3.75
4	3.68	0.15	0.24	2.15	0.89	1.82	3.77	2.30	2.55
6	4.97	0.07	0.16	1.86	1.05	1.71	4.22	4.01	3.84
24	6.19	0.78	3.07	3.32	2.45	7.09	11.39	11.88	15.83
30	6.66	2.29	3.91	5.31	3.10	9.05	14.50	15.58	20.89
S.E.	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59

Table 9b. Percent metmyoglobin means for *semimembranosus* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	3.73	1.98	7.72	2.37	2.30	0.57	1.19	3.09	2.72
1	2.97	2.18	2.86	2.40	2.05	2.49	1.64	5.09	4.92
2	3.28	2.73	1.82	3.90	2.38	1.96	4.16	6.78	9.52
4	3.54	5.26	3.14	4.19	3.79	3.85	5.18	10.93	10.59
6	4.05	5.50	4.18	5.66	17.30	4.53	9.29	15.27	13.12
24	4.03	8.30	10.34	11.72	17.39	14.98	31.41	33.88	22.41
30	7.13	12.61	10.78	14.65	24.44	22.87	34.99	36.64	26.75
S.E.	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56

Table 9c. Percent metmyoglobin means for *psoas major* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	6.39	3.73	7.24	7.29	7.17	4.70	3.81	7.14	4.92
1	6.37	5.25	3.63	5.93	5.01	4.93	7.70	8.10	11.29
2	5.90	6.33	6.91	8.33	8.57	6.59	13.03	9.16	16.49
4	7.70	11.18	8.61	9.46	8.65	10.00	19.94	16.17	28.25
6	7.98	11.29	9.76	9.42	11.08	11.81	26.26	20.13	33.03
24	9.91	23.38	26.60	23.79	30.62	32.58	53.34	42.66	56.15
30	11.08	28.58	36.29	29.00	32.17	37.99	57.36	46.58	59.15
S.E.	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61

[1]% Metmyoglobin=$\frac{(K/S\ 572 \div K/S\ 525 \text{ for } 100\% \text{ deoxy.}) - (K/S\ 572 \div K/S\ 525 \text{ for sample})}{(K/S\ 572 \div K/S\ 525 \text{ for } 100\% \text{ deoxy}) - (K/S\ 572 \div K/S\ 525 \text{ for } 100\% \text{ metmyoglobin})} \times 100$

Table 10a. Percent deoxymyoglobin[1] means for *longissimus dorsi* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	14.14	19.97	19.78	15.97	28.67	16.53	13.71	7.81	14.58
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.E.	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21

Table 10b. Percent deoxymyoglobin means for *semimembranosus* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	11.88	12.76	17.29	17.79	10.77	11.82	19.78	16.15	19.05
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.E.	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

Table 10c. Percent deoxymyoglobin means for *psoas major* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	29.86	8.92	13.37	20.16	17.67	23.26	14.97	10.73	21.16
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	7.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	6.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.E.	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34

[1]% Deoxymyoglobin=$\frac{(K/S\ 474 \div K/S\ 525 \text{ for } 100\% \text{ met.}) - (K/S\ 474 \div K/S\ 525 \text{ for sample})}{(K/S\ 474 \div K/S\ 525 \text{ for } 100\% \text{ met.}) - (K/S\ 474 \div K/S\ 525 \text{ for } 100\% \text{ deoxymyoglobin})} \times 100$

Table 11a. Percent oxymyoglobin[1] means for *longissimus dorsi* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	36.07	62.38	43.43	44.70	67.66	63.58	56.83	57.45	66.14
1	60.37	93.76	89.17	73.85	92.64	88.65	83.97	79.29	89.81
2	61.22	91.70	73.88	82.07	95.84	92.50	83.45	85.33	81.39
4	64.07	93.68	93.93	80.05	96.44	90.22	76.42	82.67	86.39
6	72.57	95.86	92.63	78.99	95.14	91.27	76.49	76.37	80.03
24	82.92	91.90	85.28	75.98	82.59	76.54	61.57	63.04	63.59
30	78.93	88.67	82.41	70.76	80.61	70.43	56.57	57.18	60.70
S.E.	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31

Table 11b. Percent oxymyoglobin means for *semimembranosus* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	20.02	49.09	31.89	47.81	51.70	56.29	57.27	50.58	56.88
1	39.50	68.31	69.06	72.18	71.72	65.00	73.89	60.84	59.90
2	34.38	67.02	74.69	72.89	73.01	70.52	69.21	60.05	63.32
4	41.62	64.82	74.59	73.01	71.63	68.28	67.51	55.42	54.34
6	44.64	68.52	75.25	71.34	58.12	69.00	57.85	49.14	50.65
24	56.00	68.51	67.11	61.44	52.10	56.26	36.05	29.10	33.79
30	51.10	60.08	65.66	58.41	52.24	45.22	33.16	26.45	28.50
S.E.									

Table 11c. Percent oxymyoglobin means for *psoas major* steaks stored at -1.5°C in 100% CO_2.

Hours on Display	Storage Time (Weeks)								
	0	3	6	9	12	15	18	21	24
0	32.28	68.52	40.86	48.87	56.16	56.39	64.70	64.84	64.93
1	50.47	79.84	81.99	76.83	80.71	76.75	76.73	74.84	68.64
2	52.86	76.45	66.86	76.34	77.71	75.77	68.28	72.82	63.12
4	50.78	68.75	75.58	75.37	74.57	70.92	59.71	62.97	51.38
6	50.48	74.73	74.35	76.49	70.31	61.90	51.89	60.51	44.05
24	52.62	61.77	53.44	54.47	49.61	46.54	21.55	37.87	20.64
30	50.23	52.20	60.06	49.25	45.94	40.98	21.27	31.70	17.71
S.E.	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42

[1]% Oxymyoglobin=$\frac{(K/S\ 610 \div K/S\ 525 \text{ for } 100\%\ \text{met.}) - (K/S\ 610 \div K/S\ 525 \text{ for sample})}{(K/S\ 610 \div K/S\ 525 \text{ for } 100\%\ \text{met.}) - (K/S\ 610 \div K/S\ 525 \text{ for } 100\%\ \text{oxymyoglobin})} \times 100$

Organoleptic Evaluation

A complete laboratory organoleptic evaluation was planned for all steaks that were still desirable in appearance and odour (according to sensory colour and odour scores) after each 30 hour retail display period. After each sampling period, all steaks were frozen and then chosen on a completely random basis to be given to the trained panelists. At this time it was noticed that all steaks that were acceptable in appearance before freezing were now discoloured to levels of greater than 50% surface discolouration. Zero week steaks (samples with no CO_2 or vacuum storage) and 3 week steaks (CO_2 and vacuum) were scored as having undesirable to moderately undesirable flavours. The off flavours that were found were described by the panelists as being lemony, livery, acid-like, sour, and rancid. It was decided that the organoleptic evaluation portion of this experiment must be discontinued due to the presence of these off flavours so early in the experiment.

Microbiological Quality of Retail Beef Steaks as a Result of Storage in CO_2

Bacteriology

Throughout the CO_2 storage intervals for both storage temperatures, *B. thermosphacta*, pseudomonads and the *Enterobacteriaceae* were undetectable. Lactic acid bacteria were the only bacteria detected during extended CO_2 storage for up to 24 weeks. Data in Table 12 shows the effects of time in CO_2, storage temperature, retail display time and retail display temperature on bacterial growth of beef steaks. Under the higher retail display temperature of 6.3°C the lactic acid bacteria reach maximum cell numbers (log bacteria/cm^2 = 6 to 7) within 6 weeks of CO_2 at -1.5°C and 2.0°C. Bacteria grew faster at 2.0°C and overall numbers of lactics were slightly higher on steaks stored at 2.0°C compared to -1.5°C, but this difference was not significant ($P>0.05$). The lactic acid bacteria recovered from *longissimus dorsi* steaks that were subjected to colder retail storage conditions (2.3°C) reached maximum cell numbers at 0 weeks plus 8 days of retail display.

The lactic acid bacteria were the dominant group of organisms throughout each ten day retail display interval. Figures 1, 2 and 3 show the effects of CO_2 storage time (weeks) on the numbers of lactic acid bacteria from *longissimus dorsi* steaks recovered during retail display. Bacterial growth was not significantly effected by storage temperature ($P>0.05$) but the graphs clearly show that the lactic acid bacteria reach higher cell numbers quicker at the higher storage temperature (2.0°C, Figure 2) especially at 0 days for 6 and 12 week steaks. The numbers of recoverable lactic acid bacteria are significantly lower ($P<0.05$) at 0 weeks for 0, 2, 4, 6 and 8 days

Table 12. Effects of time in CO_2 retail display time and retail display temperature on mean bacterial growth of lactic acid bacteria.

Weeks in CO_2	Days on Retail Display	-1.5°C With Retail of 6.3°C	2.0°C With Retail of 6.3°C	2.0°C With Retail of 2.6°C
0	0	2.00[1]	2.06	2.00
0	2	2.92	2.60	4.27
0	4	3.69	3.52	4.83
0	6	3.36	3.79	5.01
0	8	3.03	3.36	6.42
0	10	5.84	3.76	6.15
6	0	4.50	6.12	6.51
6	2	5.87	6.52	6.43
6	4	6.71	6.24	7.12
6	6	7.32	7.54	7.28
6	8	7.37	7.37	7.05
6	10	7.29	7.14	7.77
12	0	6.46	6.54	6.13
12	2	6.51	7.09	6.95
12	4	6.98	7.10	7.16
12	6	7.09	6.78	7.24
12	8	7.56	7.48	7.33
12	10	7.37	7.40	7.16
18	0	6.68	6.78	5.54
18	2	7.68	7.60	6.62
18	4	7.49	7.52	7.00
18	6	6.30	7.41	7.65
18	8	7.79	7.69	7.57
18	10	6.49	7.14	7.85
24	0	5.38	7.09	NA[2]
24	2	6.74	7.01	NA
24	4	7.09	6.62	NA
24	6	6.32	5.67	NA
24	8	6.98	6.25	NA
24	10	7.48	7.58	NA

[1]Each mean in the log number of bacteria/cm^2.

[2]Data is not available. Steaks were only stored for 18 weeks at these temperatures.

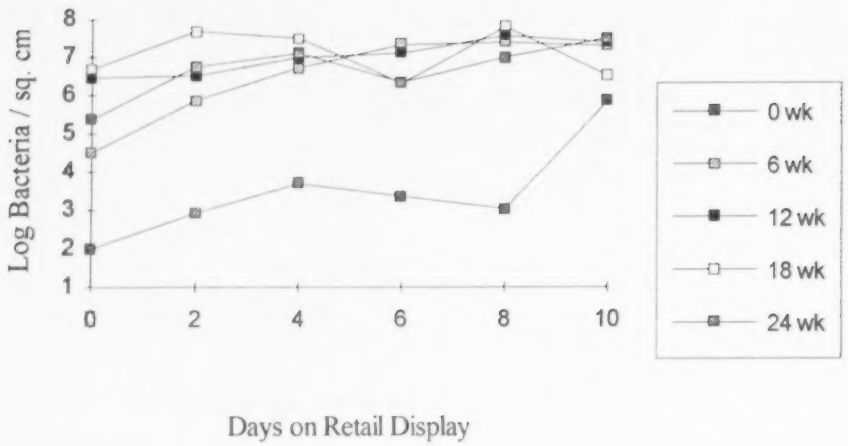

Figure 1. Effects of carbon dioxide storage time at -1.5 degrees C and days on retail display on lactics recovered from retail steaks (6.3 degrees C). Each point represents the LSM of 5 steaks.

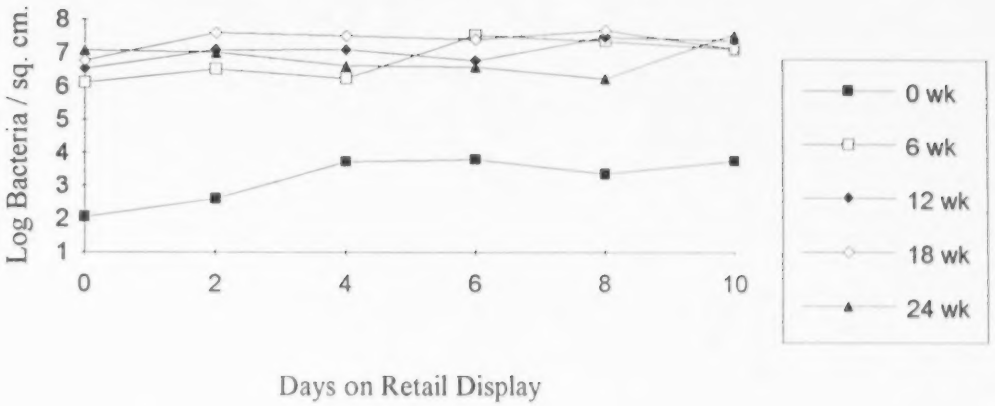

Figure 2. Effects of carbon dioxide storage time at 2.0 degrees C and days on retail display on lactics recovered from retail steaks (6.3 degrees C). Each point represents the LSM of 5 steaks.

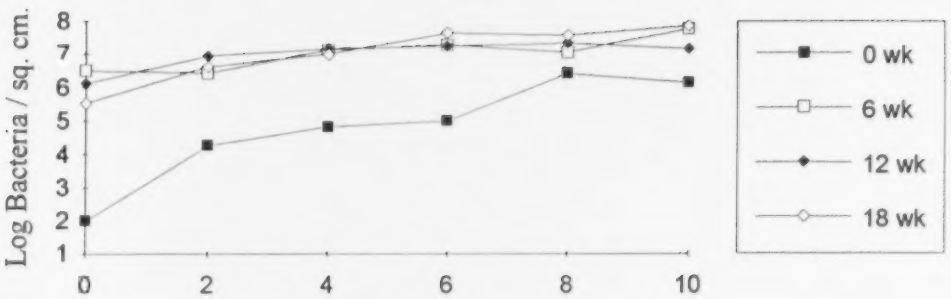

Figure. 3. Effects of carbon dioxide storage time at 2.0 degrees C and days on retail display on lactics recovered from retail steaks (2.6 degrees C). Each point represents the LSM of 5 steaks.

of retail display at both storage temperatures compared to all other storage times (weeks). Retail display temperature had no significant effect on numbers of bacteria ($P>0.05$). In fact, numbers of lactics reached maximum densities quicker at the colder retail temperature, especially at 0 weeks. Increased time in CO_2 resulted in an increase in the numbers of lactic acid bacteria recovered during retail display.

The only other detectable bacterial population during retail display were the pseudomonads. Figures 4, 5 and 6 show the effects of CO_2 storage time and days on retail display on the numbers of pseudomonads recovered from retail steaks. The pseudomonads grew slowly after a lag period of 4 days to reach maximum cell numbers of 6.89 log bacteria/cm^2 for 0 week steaks (Figure 4) and 7.18 log bacteria/cm^2 for 0 weeks steaks (Figure 5). At the colder retail storage temperature of 2.6°C, 0 week steaks reached maximum cell numbers of 3.27 log bacteria/cm^2 (Figure 6). As CO_2 storage time increased there was a significant decrease ($P<0.05$) in the numbers of detectable pseudomonads and this decrease was greater after CO_2 storage at 2.0°C (Figure 5) than at -1.5°C (Figure 6) ($P<0.05$). At -1.5°C, pseudomonads were reduced to undetectable levels (log bacteria /cm^2 = <2.00) at 18 weeks (Figure 4) storage. At the higher CO_2 storage temperature of 2.0°C, the pseudomonads were undetectable at 12 weeks storage. Steaks stored at 2.0°C followed by the colder retail display temperature of 2.6°C, showed that the pseudomonads were undetectable at 6 weeks. Thereafter, no pseudomonads could be recovered from retail steaks at any retail display time.

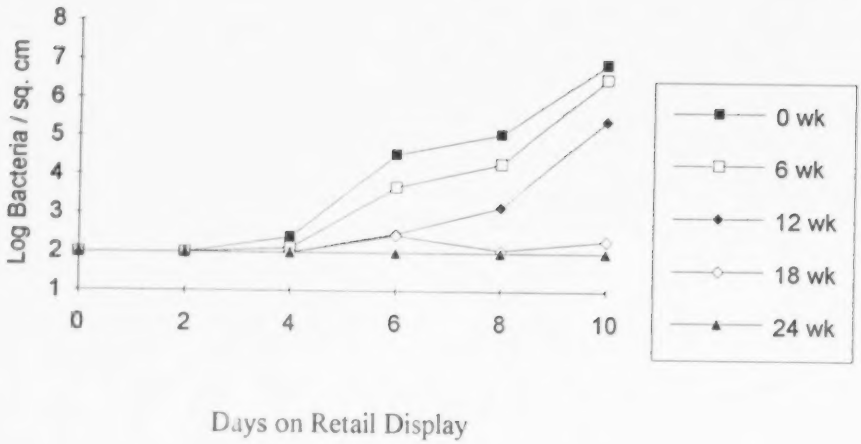

Figure 4. Effects of carbon dioxide storage time at -1.5 degrees C and days on retail display on pseudomonads recovered from retail steaks (6.3 degrees C). Each point represents the LSM of 5 steaks.

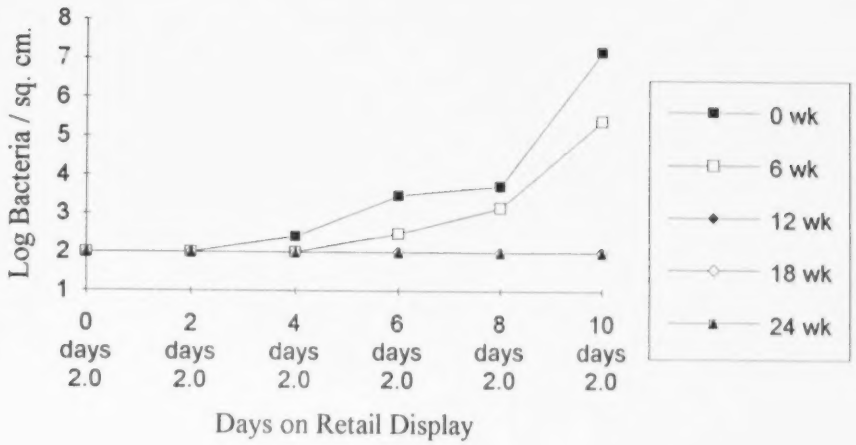

Figure 5. Effects of carbon dioxide storage time at 2.0 degrees C and days on retail display on pseudomonads recovered from retail steaks (6.3 degrees C). Each point represents the LSM of 5 steaks.

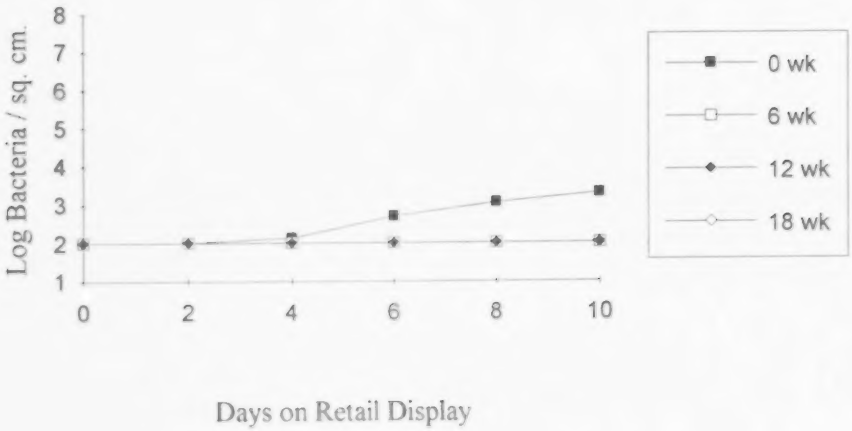

Figure 6. Effects of carbon dioxide storage time at 2.0 degrees C and days on retail display on pseudomonads recovered from retail steaks (2.6 degrees C). Each point represents the LSM of 5 steaks.

Sensory Colour, Discolouration and Odour Intensity

The effects of time in CO_2 and days on retail display for both storage temperatures and both retail display temperatures on *longissimus dorsi* steaks colour and discolouration means is illustrated in Table 13. There were no significant differences on sensory colour or discolouration means between the two storage temperatures nor between the two retail display temperatures ($P>0.05$). Throughout the 0 week storage interval there were no significant differences ($P>0.05$) between steaks according to retail display temperature for colour and discolouration means. For each 6 week storage time/10 day retail display interval, the colour scores increased (the steaks became darker red) and the amount of surface discolouration increased. All steaks were rated as being completely discoloured after 24 weeks storage on 6, 8 and 10 days of retail display from both storage temperatures. At the colder retail storage temperature of 2.6°C, the steaks were completely discoloured at 12 week storage plus 8 days of retail display.

Off odour intensity means are shown in Table 14. With increasing storage time (weeks) and days on retail display there is a significant increase ($P<0.05$) in the off odour intensity (off odour becomes more prevalent). No significant difference ($P>0.05$) is found for off odour means when comparing the two storage temperatures (-1.5°C and 2.0°C) or when comparing the two retail display temperatures (6.3°C and 2.6°C).

The effects of CO_2 storage time, storage temperature and retail display temperature upon retail case life (days) are shown in Table 15. The retail case life of *longissimus*

Table 13. Effects of time in CO_2 and days on retail display on colour and discolouration of *longissimus dorsi* steaks.

		6.3°C Colour		6.3°C Discolouration		2.6°C Colour	2.6°C[2] Discolouration
Storage Time(Weeks)	Retail Display (Days)	-1.5°	2.0°	-1.5°	2.0°	2.0°	2.0°[3]
0	0	5.0[1]	5.0	1.0[3]	1.0	5.1	1.0
0	2	5.3	5.3	1.1	1.0	5.3	1.0
0	4	5.7	5.6	2.2	1.8	5.9	2.1
0	6	5.6	5.4	2.6	2.4	6.5	3.8
0	8	5.6	5.9	4.2	3.8	7.0	3.8
0	10	6.1	6.1	6.4	5.5	6.9	5.3
6	0	5.6	5.7	1.0	1.0	4.8	1.0
6	2	5.8	6.1	2.8	4.2	5.1	4.9
6	4	6.1	6.1	5.4	4.7	5.7	5.3
6	6	6.5	6.5	6.0	6.2	6.8	6.7
6	8	6.4	7.3	6.4	6.8	6.7	6.8
6	10	8.0	6.2	7.0	6.8	6.8	6.9
12	0	5.4	5.2	1.0	1.0	5.5	1.0
12	2	5.8	6.4	2.8	3.4	6.3	3.2
12	4	6.4	5.8	5.5	5.6	7.1	5.6
12	6	6.3	6.3	6.6	6.6	7.6	7.0
12	8	7.0	5.8	6.8	6.7	CD	7.0
12	10	6.6	7.0	6.8	6.9	CD	7.0
18	0	5.4	5.0	1.0	1.0	5.3	1.1
18	2	5.9	6.0	2.9	5.0	6.1	3.6
18	4	6.8	6.9	5.0	6.1	6.8	5.9
18	6	6.7	5.7	5.5	6.2	CD	7.0
18	8	5.8	6.7	6.8	6.6	CD	7.0
18	10	7.0	7.2	6.4	7.0	CD	7.0
24	0	5.5	5.0	1.0	1.5	NA[5]	NA
24	2	6.0	6.3	3.4	4.9	NA	NA
24	4	6.0	6.4	4.9	6.1	NA	NA
24	6	CD[5]	CD	7.0	7.0	NA	NA
24	8	CD	CD	7.0	7.0	NA	NA
24	10	CD	CD	7.0	7.0	NA	NA
	SE=	0.17	0.16	0.31	0.32	0.17	0.33

[1]Represents the mean of 5 steaks. Colour scores are based on a 9-point descriptive scale (0=completely discoloured, 1=white and 8=extremely dark red).
[2]Retail temperature
[3]CO_2 storage temperature
[4]Represents the mean of 5 steaks. Percent discolouration scores are based on a 7-point descriptive scale (1=0% surface discolouration and 7=100% surface discolouration).
[5]CD=Completely discoloured.
[6]Data is not available. Steaks were only stored under CO_2 for 18 weeks in Part 2.

Table 14. Effects of time in CO_2 and days on retail display on off odour intensity of *Longissimus dorsi* steaks.

		6.28°C[2] Odour Intensity		2.6°C Odour Intensity
Storage Time (Weeks)	Retail Display (Days)	-1.5°C	2.0°C	2.0°C[3]
0	0	1.0[1]	1.0	1.0
0	2	1.0	1.1	1.0
0	4	1.5	1.3	1.2
0	6	1.7	1.5	1.5
0	8	1.9	1.7	1.9
0	10	3.4	2.8	3.1
6	0	1.2	1.9	1.5
6	2	1.8	2.9	2.0
6	4	2.8	3.3	2.7
6	6	3.2	3.4	3.4
6	8	3.2	3.4	3.7
6	10	3.4	3.5	3.7
12	0	1.7	2.5	1.2
12	2	1.5	2.4	1.7
12	4	2.1	3.2	1.8
12	6	2.8	3.4	2.8
12	8	3.1	3.1	3.2
12	10	3.4	3.8	3.6
18	0	1.4	1.8	1.9
18	2	1.8	3.3	2.5
18	4	1.9	3.3	2.9
18	6	2.9	3.4	3.1
18	8	3.3	3.6	3.4
18	10	3.4	3.8	3.6
24	0	1.5	1.6	NA[4]
24	2	1.6	1.7	NA
24	4	2.2	3.3	NA
24	6	3.0	3.8	NA
24	8	4.1	3.9	NA
24	10	3.6	3.7	NA
	SE=	0.17	0.15	0.14

[1]Represents the mean of 5 steaks. Odour intensity scores are based on a 4-point descriptive scale (1=no off odour and 4=prevelant off odour).
[2]Retail storage temperature.
[3]CO_2 storage temperature.
[4]Data is not available. Steaks were only stored under CO_2 for 18 weeks in Part 2.

Table 15. Effect of time in CO_2 storage(weeks) on retail case life.

Storage Time in CO_2 (Weeks)	Appearance Case Life (Days) 6.3°C -1.5°C	Appearance Case Life (Days) 6.3°C 2.0°C	Appearance Case Life (Days) 2.6°C 2.0°C	Odour Case Life (Days) 6.3°C -1.5°C	Odour Case Life (Days) 6.3°C 2.0°C	Odour Case Life (Days) 2.6°C 2.0°C
0	5.8a[1]	6.9a	8.0a	8.8a	9.6a	9.9a
6	2.2b	1.6c	2.4b	3.5e	2.9b	3.1b
12	2.3b	2.0b	2.4b	4.1d	2.8b	3.2b
18	2.2b	1.4d	1.5c	4.7c	1.4c	2.8c
24	1.9c	1.1e	NA[2]	5.2b	1.4c	NA

[1]Least squares means within the same column bearing the same letter are not significantly different (P>0.05).
[2]Data is not available. Steaks were only stored under CO_2 for 18 weeks in Part 2.

dorsi steaks decreased, for both storage temperatures, as storage time in CO_2 increased ($P<0.05$). Appearance deteriorated more quickly than odour. During the first storage interval (retail display temperature of 6.3°C) (0 to 6 weeks) case life of the steaks was reduced by as much as 60% at -1.5°C, and 75% at 2.0°C. At the colder retail display temperature (retail display temperature of 2.6°C), the appearance case had deteriorated by 70% during the first 6 week storage interval.

Discussion

Acceptability of Different Colour Stability Muscles After Storage in 100% CO_2

The sensory panel colour mean scores remained quite constant throughout the 24 week storage interval. At the storage temperature of -1.5°C (the temperature which has the greatest oxygen penetration (Kropf *et al.*, 1985)), most of the samples remained bright cherry red in colour. At higher storage temperatures , the globin of oxymyoglobin becomes less efficient in protecting the heme (Seideman *et al.*, 1984) and unstable deoxymyoglobin formation is favoured (Walter, 1975). Once the steaks were exposed to air, colour deterioration from red to brown began to occur (Kropf *et al.*, 1985)

Differences in muscle type had a significant effect on meat colour with the *longissiums dorsi* steaks showing the greatest colour stability and the *psoas major* steaks showing the least colour stability. Similar results were found in by O'Keefe and Hood (1980-81a) Ledward(1985) and Renerre (1990). Packaging and display hour had no significant effect on beef colour scores, which is in agreement with anoxic storage data reported for retail ready pork chops (Scholtz *et al.*, 1992b).

The data presented show no significant variations from the colour score of 5 (bright cherry red). There are a number of possible explanations for this observation. First of all, light from the retail environment has a dramatic effect on overall colour, but the human eye is not sensitive enough to detect these changes (Kropf *et al.*, 1980).

The incandescent lights altered the meat pigments and changed the overall colour, as measured by instruments, but the sensory panel could not distinguish these changes.

Present data support previous findings that the amount of surface discolouration increases as storage time in CO_2 increases (Gill, 1986; Shay and Egan, 1990). The amount of surface discolouration (according to sensory panel) increases significantly with increases in CO_2 storage time.

Steaks from all three muscle types evaluated at 0 weeks storage had greater mean discolouration scores than steaks evaluated at 3 and 6 weeks of storage. These 0 week steaks did not appear fresh at the onset of the experiment. Again, *longissiums dorsi* steaks were the most colour stable (O'Keefe and Hood, 1980-81a) and showed the least amount of surface discolouration as time in CO_2 storage increased.

Retail acceptance is the ultimate determinant of any preservative/processing system (Jeremiah, 1982; Muller, 1990) and colour is the most important factor in affecting consumer acceptance (Jeremiah, 1982; Agullo *et al.*, 1990). Several researchers have reported that extended anoxic storage of chilled meats causes a reduction in retail acceptability under aerobic conditions (Moore and Gill, 1987; Shay and Egan, 1990). In the current study, retail acceptance means decreased significantly with increased CO_2 storage time (weeks). Not unexpectedly, the results of these experiments show that steaks from all three muscles had significant decreases in retail acceptability from 0 to 24 weeks. *Longissimus dorsi* steaks were the most acceptable for longer periods of storage time. However, retail ready beef steaks from *longissimus dorsi* and *semimembranosus* muscles stored under anoxic conditions can be kept for 24 weeks at -1.5°C and still be of acceptable appearance, which is in agreement with other studies with pork (Spahl *et al.*, 1981; Gill and Harrison, 1989) and beef (Gill and Penney, 1988).

The results presented thus far clearly support the results of others that low temperatures improve the inhibitory effects of CO_2 (Enfors and Molin, 1981; Finne, 1982), improve colour and retail acceptability, decrease surface discolouration and retard off odour development. These findings support the conclusion that the main factor affecting colour and shelf life of steaks is temperature (Greer and Jeremiah, 1980; Powell and Cain, 1987).

Present results also support the fact that steaks which have been stored in anoxic atmospheres must be transferred to aerobic conditions so the meat can "bloom" to the desirable cherry red colour (O'Keefe and Hood, 1980-81a; Egbert and Cornforth, 1986; Rousset and Renerre, 1990). Consumers will reject beef and rate it as being unacceptable if it contains as little as 20% of the brown pigment (MacDougall, 1982; Renerre and Mazuel, 1985).

Current findings revealed that off odour intensity did not change with muscle type, but increased with increasing anoxic storage time. Odours remained acceptable for all muscle type/retail display conditions after 21 weeks storage at -1.5°C. These results agree with an anoxic study of beef storage reported by Gill and Penney (1988). The CAP system seems to eliminate any volatile odour which may be offensive (Gill and Penney, 1988). Sensory data from panelists in this study showed that very few steaks had putrid off odours.

The pH decreased significantly as the experiment progressed. This observation agrees with previous research (Nassos *et al.*, 1983; Egan and Shay, 1988; Seman *et al.*, 1989; Brody, 1989b; Rousset and Renerre, 1990) which also concluded that pH decreased as storage time increased. A study of vacuum packaged round steaks (Hanna *et al.*, 1983) found a marked decrease in pH (0.2 to 0.5 units) as storage time increased. However, the present work is the first to report a pH below 5.0 after

extended anoxic storage. The pH probably decreased due to the high bacterial population of lactics, which produce lactic acid as a fermentative end-product. Also, packaging fresh meat in 100% CO_2 can cause the pH to decrease due to CO_2 absorption and ionization of carbonic acid (Brody, 1989b). It is conceivable that the combination of these two factors during extended periods of anoxic storage could have decreased the pH to these low levels. Low pH is detrimental to meat quality, since a reduction in pH can contribute to increasing surface discolouration as storage time increases. As well, low pH causes the globin moiety to denature and dissociate from the heme (Seideman *et al.*, 1984).

Present results show that L* mean scores (light-dark axis) did not change significantly throughout the 24 week storage interval for *longissimus dorsi* and *psoas major* steaks. This agrees with the colour data conclusions presented previously, but disagrees with results from CO_2 stored lamb (Moore and Gill, 1987) where researchers found that there was a significant increase in L* values (the meat was lighter in colour) as storage time increased.

Mean a* values (red-green axis) for all three muscle types decreased (steaks were less red) as CO_2 storage time increased. This may explain the sensory panel results which show that as time progresses there was an increase in surface discolouration (browning), ultimately causing the steaks to appear less red. Present results agree with those of Fu *et al.*, 1992. These workers found that beef rib eye steaks, packaged in high CO_2 atmospheres, had significantly lowered a* values as storage time increased.

The data obtained show no significant differences in mean b* values (yellow-blue axis) for *longissimus dorsi* and *semimembranosus* steaks. *Psoas major* steaks appear to be less stable. Mean b* scores have been reported to increase during anoxic storage (Moore and Gill, 1987) indicating an increase in yellow colour.

The results presented for pigment percentages clearly show an increase in metmyoglobin as storage time or retail hour increase. Other studies on retail ready beef reported the same results (Echevarne *et al.*, 1990, Rousset and Renerre, 1990; Rousset and Renerre, 1991). Fresh meat has a limited capacity to convert metmyoglobin back to deoxymyoglobin. Once the production of metmyoglobin (which increases with storage time) exceeds the meat's enzymic reducing system, the meat surface will become permanently brown (Penney and Bell, 1993). As well, a low pH environment favours oxidation of the myoglobin molecule to metmyoglobin, resulting in more rapid discolouration of fresh meats (Cole, 1986). The *psoas major* steaks had the greatest mean percentages of metmyoglobin and *longissimus dorsi* steaks had the lowest amounts of metmyglobin. This was expected because individual muscles differ in their colour stability (O'Keefe and Hood, 1982; Renerre, 1990). Muscles like the *psoas major*, which have high rates of oxygen consumption, also have the fastest rates of metmyoglobin formation (O'Keefe and Hood; Renerre and Labas, 1987).

When storage time and display hour were evaluated, significant differences in deoxymyoglobin were found for retail display time in hours. Immediately after removal from the anoxic conditions of the foil laminate pouch, all steaks have myoglobin in the reduced pigment form (deoxymyoglobin). Beef packaged in CAP appears to be extremely stable and its myoglobin must be predominately in the deoxymyoglobin form (Gill, 1990). Due to the lack of oxygen, no oxidation reactions could occur, so deoxymyoglobin dominates (Gill, 1990). Once the steaks are exposed to the air, reduction begins to occur immediately, and results in a dramatic decrease in the amount of detectable deoxymyoglobin (Kropf *et al.*, 1985). The present results

agree with previous work (Finne, 1982; Taylor *et al.*, 1990) in that meat colour is very unstable once the meat is transferred to an aerobic environment.

Present results for percent oxymyoglobin show significant decreases as storage time in CO_2 progresses. Oxymyoglobin levels decrease with time and metmyoglobin concentration increases at or near the surface (Kropf *et al.*, 1985). The most colour stable muscle, *longissimus dorsi*, produces retail ready steaks that have significantly more oxymyoglobin than the other two muscles. Increases in retail display time decreased the amount of detectable oxymyoglobin. As time on retail display increases the oxygen permeability of the overwrap film decreases and the meat's oxygen-utilizing enzymes demand more oxygen (Powell and Cain, 1987).

Previous studies with pork have shown that sour flavours were not detected until 4-5 weeks (Gill and Harrison, 1989) and after 9 weeks (Greer, 1992) storage when the numbers of lactic acid bacteria had reached populations of $10^7/cm^2$. Vacuum-packaged beef developed off-flavours described as "liver-like", sour, acid and bitter (Egan and Shay, 1982). Beef stored in CO_2 develops strong acid/putrid flavours by week 12 at 1.0°C (Gill and Penney, 1988). These attributes are similar to those described in the present study although current results are of a preliminary nature and not conclusive.

Spoilage by lactic acid bacteria is characterized by acidic fermentation products described as acid and sour (Egan, 1983) and these can be of major concern (Dainty and Mackey, 1992). It is also speculated that lipid oxidation is one of the major causes of raw meat quality deterioration (Raharjo and Sofos, 1993) and myoglobin may act as a catalyst for this reaction.

Microbiological Quality of Retail Beef Steaks as a Result of Storage in CO_2

Present results show that the only detectable bacterial population throughout the 24 week storage interval (-1.5°C and 2.0°C) in CO_2 at both retail storage temperatures was the lactic acid bacteria. Predictably, the numbers of bacteria were lower at the lower retail storage temperature. Studies with vacuum packaged beef have shown no consistent differences in the microflora as a result of retail storage temperature (Vanderzant *et al.*, 1982). Thus, the growth of *Brochothrix thermosphacta* and *Enterobacteriaceae* were completely inhibited. Similar results have been shown with CO_2 packaged pork (Egan and Roberts, 1987; Greer *et al.*, 1992; Greer *et al.*, 1993) and beef (Vanderzant *et al.*, 1982; Newsome *et al.* 1984; Rousset and Renerre, 1990).

There are a number of factors contributing to the dominance of the lactic acid bacteria in this study. Present data support previous conclusions that high concentrations of CO_2 were very effective in inhibiting the growth of potent spoilage organisms (Shay and Egan, 1987; Gill and Harrison, 1989) allowing the CO_2-resistant lactic acid bacteria to thrive (Blickstad and Molin, 1983). At low storage temperatures (-1.5°C), *B. thermosphacta* is totally inhibited by CO_2 (Gill and Harrison, 1989). The anoxic environment produced by packaging the meat with the Captech process (Gill, 1989) inhibits growth of aerobic spoilage organisms and allows the growth of the facultative anaerobes, the lactic acid bacteria (Brody, 1989b). It has also been well established that certain lactic acid bacteria produce antimicrobial compounds called bacteriocins (Gill, 1982; Ahn and Stiles, 1990)as well as hydrogen peroxide and lactic acid that inhibit the growth of certain undesirable spoilage organisms and *B. thermosphacta* (Dainty and Mackey, 1992).

The change in pH during storage may also play a role in the survival and dominance of the lactic acid bacteria, as low pH meats will develop a flora consisting of only lactic acid bacteria (Gill and Molin, 1991). It can be speculated, based on the pH results from the first experiment, that as storage time in CO_2 increased, the surface pH decreased. A decrease in PH was also found in previous anoxic storage studies (Nassos *et al.*, 1983; Hanna *et al.*, 1983; Seman *et al.*, 1989). The lactic acid bacteria can survive and grow at lower pH and the other bacterial population are more pH sensitive. A reduction in pH causes some microorganisms to have higher growth temperatures (Kraft, 1986). Also pH plays a significant role on the growth of *B. thermosphacta* under anaerobic conditions as the bacteria will not grow below pH 5.8 in anoxic conditions (Dainty *et al.*, 1983; Dainty and Mackey, 1992).

More importantly, some interesting changes occurred when beef steaks were removed from anoxic storage in CO_2 and subjected to the aerobic conditions of retail display. Present data agree with previous results on pork (Spahl *et al.*, 1982; Scholtz *et al.*, 1992a; Scholtz *et al.*, 1992b) that the inhibitory effects of CO_2 continue to function and are expressed once transferred to air. For both retail storage temperatures, the lactic acid bacteria remained dominant throughout each 10 day display interval with the exception of 0 week steaks. Steaks that had not been subjected to CO_2 storage showed a dominance of pseudomonads during the 10 day retail display period which is to be expected under completely aerobic conditions (Nychas and Arkoudelos, 1990). The dominance of lactic acid bacteria during retail display supports results determined with CO_2 packaged pork (Scholtz *et al.*, 1992a; Greer, *et al.*, 1992; Greer *et al.*, 1993). Pseudomonads recovered from beef steaks that had undergone anoxic storage from 6 to 24 weeks, emerged as the only other detectable group of bacteria at the retail level at both retail storage temperatures.

However, their numbers were always lower than the lactic acid bacteria, which is in agreement with results reported with pork (Greer *et al.*, 1993).

As CO_2 storage time increased at both storage temperatures of -1.5°C and 2.0°C, the numbers of pseudomonads recovered from steaks during retail display decreased significantly until they were no longer detectable. The inhibition of meat spoilage bacteria, including pseudomonads, by CO_2 supports previous findings (Gill and Tan, 1980; Newsome *et al.*, 1984). However, this is the first known observation that extended storage of beef in CO_2 reduces the numbers of pseudomonads recovered from steaks during subsequent retail display under aerobic conditions. Furthermore, this effect was more pronounced at the higher CO_2 storage temperatures. This is contradictory to reports of inhibitory effects of CO_2 increasing with decreasing storage temperatures (Egan and Molin, 1981). Apart from CO_2, it is conceivable that increasing numbers of lactic acid bacteria during anoxic storage antagonize the pseudomonads and mediate a reduction in their numbers (Dainty and Mackey, 1992). On a surface model system, a combination of CO_2 and a decrease in pH seem to have a synergistic inhibitory effect on the growth rate of pseudomonads (Eyles *et al.*, 1993).

Present findings may have important implications regarding the aerobic shelf-life of retail beef ready beef after storage in CO_2. Through the elimination of the aerobic spoilage organisms, pseudomonads, and the dominance of lactic acid bacteria, the usual putrid spoilage odours may be replaced by a delayed souring (Egan and Shay, 1982; Egan, 1983).

Present results show that increases in storage time (weeks) and days on retail display cause retail ready beef steaks to become more dark red in colour due to the increases in the brown pigment, metmyoglobin. Similar conclusions were also drawn with experiments incorporating extended CO_2 storage plus retail display with venison

(Seman *et al.*, 1989) and pork (Buys and Nortje, 1993). The amount of surface discolouration also increases significantly with time, conceivably due to a reduction in metmyoglobin reducing activity (Stewart *et al.*, 1965; Ledward, 1985). Colour also can deteriorate with increasing storage time due to the decrease in pH. PH values less than 5.4 accelerate pigment autoxidation, increasing the conversions of oxymyoglobin to deoxymyoglobin to metmyoglobin (Pirko and Ayres, 1957; Livingston and Brown, 1981).

With increases in CO_2 storage time, storage temperature and retail display temperature retail case life of the steaks decreased significantly. Appearance case life deteriorated more quickly than odour for all beef steaks. Contradictory results were found with pork (Spahl *et al.*, 1981) where odour became objectionable before the meat was rejected based on appearance. There has been only limited work on the aerobic bacteriology and case life of beef steaks after storage for extended periods of time in 100% CO_2. Previous research found retail ready steaks packaged and stored in CO_2 had significantly more surface discolouration, decreased appearance ratings and higher bacterial numbers in comparison to steaks derived from vacuum packaged primals (Seideman *et al.*, 1980; Christopher *et al.*, 1980). In contrast to these earlier reports, the current study would complement recent data of Rousset and Renerre (1990), who reported CO_2 could maintain quality and improve case life of retail steaks. That is, retail ready beef steaks can be stored for up to 12 weeks in CO_2 at -1.5°C or 2.0°C and still have a retail case life of 2 days which is comparable to that presently acheived under retail conditions without subsequent anoxic storage.

In summary, the results of this study show that temperature (both throughout CO_2 storage and during retail display), time of CO_2 storage and time in the retail environment all have significant effects on the quality of retail displayed steaks after

CO_2 storage in the CAPTECH system. All of these parameters must be carefully controlled in order to provide a retail ready product that is of the highest quality in terms of appearance, bacteriology, case-life and eating quality. It does not seem practical to produce a retail ready product that can be stored for 18-24 weeks under CO_2, only to have it deteriorate in less than 24 hours once subjected to the aerobic conditions of retail display. However, keeping the meat at -1.5°C was an effective means to minimize deteriorative changes. The results of this experiment do conclude that retail ready beef steaks can be stored in 100% CO_2 for 9-12 weeks and still be acceptable once placed onto retail display. This has important implications for the domestic and export market-place. Controlled atmosphere packaged beef can safely be transported to different areas and still be of high quality in terms of colour and microbial condition.

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